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THE BEHAVIOR OF *TOXOPLASMA* WITH NOTES ON ITS TAXONOMIC STATUS[1]

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Because toxoplasmosis is now known to be a common human infection, and sometimes a very serious one, it is receiving an increasing amount of study. The organism itself, however, has had surprisingly little careful attention. This is evidenced by the striking differences in the way in which the subject is treated by authors of many of the standard texts in parasitology and protozoology. There is general agreement that *Toxoplasma* is probably a protozoan, but little as to what kind of a protozoan it may be.

Kudo (1950), for example, put *Toxoplasma* with the BABESIIDAE, one of 3 families in the order HAEMOSPORIDIA. Since the other 3 genera of the family are all parasitic in the erythrocytes of vertebrates, and two of the three are known to be tick-transmitted, this disposition of *Toxoplasma* seems at least illogical. Belding (1952) remarked (p. 240) "Flagella have been observed in a few specimens," thus, by implication at least, suggesting that the genus should be placed with the MASTIGOPHORA, since true flagella do not occur at any stage of the sporozoan life-cycle. He nevertheless included it with "Unclassified Sporozoa."

Toxoplasma receives similar treatment in most other currently used texts in protozoology and parasitology. In some, the authors are even more non-committal, placing the parasite under a heading such as "Parasites Of Doubtful Nature." Chandler (1949) went a little farther than most in stating "It is uncertain whether it is a protozoan or a fungus, although it is more probably the former." There are still authors who omit it altogether.

Very lately the suggestion has again been made that the real affinities of this parasite may be with the SARCOSPORIDIA. Biocca (1949) remarked on the fact that, although *Toxoplasma* and *Sarcocystis* have certain characteristics in common, they are easily distinguishable. Piekarski (1950) took essentially the same position, but Muhlpfordt (1951) has apparently shown that the Sabin-Feldman dye test gives positive results both with toxoplasmosis and sarcosporidiosis. It has long been recognized that many human cases diagnosed as the latter condition were really the former. The problem was considered in some detail by Kean and Grocott (1945), and Biocca (1949) went so far as to claim that probably half the reported human cases of sarcosporidiosis were in fact toxoplasmosis.

The case is further complicated by the existence of other species of parasites

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closely resembling those in these two genera. *Encephalitozoon,* for example, is morphologically much like *Toxoplasma.* Wenyon (1926) called attention to marked similarities, and Biocca (1949) concluded that a third genus, *Soroplasma,* also had so much in common with these two that the three should be combined under the single generic name *Toxoplasma.*

Because the morphology and behavior of the parasite seem very important in settling its true biological position, and the literature contains a good many conflicting statements, we undertook a careful restudy of the organism itself, especially from the point of view of the motility and the possible presence of flagella. Splendore (1913a) was probably the first to describe movement by *Toxoplasma,* and he also claimed to have seen flagella. Since that time the parasite has been said to be capable of independent movement by some, and to be non-motile by others. Splendore believed he saw a single flagellum, but Cross (1947) saw two in certain specimens from a single mouse, though she conceded the need for confirmation. Most observers, even when they saw movement of the organism, were unable to see any flagella. Nor have any of them apparently made detailed, careful studies of the behavior of *Toxoplasma.*

MATERIALS AND METHODS

We used the R. H. strain of *Toxoplasma,* a stock originally derived from a human case by Sabin in 1940 (Sabin, 1941), and supplied to us several years ago through the courtesy of Dr. Harry Feldman. Since that time we have maintained it in albino mice, usually by serial passage of infected peritoneal exudate. It has also been transferred to a number of species of domestic and wild birds at different times, and from them transmitted back to mice, but without causing any noticeable changes in the number required to infect, virulence, or morphology and behavior.

For study of organisms under the microscope, heavily parasitized peritoneal exudate was used. This was either examined directly, or in some cases diluted with physiological saline to facilitate observation of individual parasites.

For actual study three methods were employed: after covering with a No. 1 cover slip, the preparation was examined, usually under oil, either on an ordinary stage, or a constant temperature (37° C.) warm stage under bright field illumination, or with the dark field, since this latter is especially favorable for the demonstration of flagella. The magnification varied from 660× to 1455×. Although cooling to room temperature did not seem to have much effect on the behavior of the parasites, observation on the warm stage proved the better method, since the exudate of such preparations dried rather quickly around the edge of the cover glass, thus sealing it from further desiccation. Such slides could often be studied for as long as 3 hours at a sitting. The dark field proved superior when studying the behavior of *Toxoplasma* with reference to leucocytes or other host cells in the exudate.

Since we were especially anxious to settle, if possible, the disputed question of the existence of flagella, we also made preparations according to Leifson's method (Leifson, 1930), using organisms in peritoneal exudate and (after washing and centrifugation) in a suspension of physiological saline. Although this stain was devised primarily for showing bacterial flagella, there seemed no reason why it should not be also successful with organisms such as *Toxoplasma.*

OBSERVATIONS

Despite the difficulty which some observers have had in detecting motility in *Toxoplasma,* it was soon found that the organisms were quite active. A puzzling fact however was their different behavior in different preparations. On slides from certain exudates, little or no movement could be seen, but in others motion was much more evident.

Since the exudate was always taken from acutely ill mice (inoculated 3 to 7 days earlier), it seemed unlikely that differences in the stage of the infection could be in any way responsible—especially as mice develop little immunity, and do so slowly. Nor did it seem that differing conditions of observation could be in any way responsible.

Another unexplained fact was the relatively small percentage of parasites which usually exhibit activity in any given preparation. But there could be no doubt about the motility of these, although it often required several minutes of observation before any of them could be caught in the act. And sometimes a preparation was found in which the majority of the organisms were quite active.

A number of different kinds of behavior were observed. The organisms often appeared to somersault, diving into the medium anterior (pointed) end first[3], and then returning to the original plane of focus. No distance is gained by this manoeuver, and what its advantage to the parasite may be can only be guessed. It would seem to be a rather fatiguing exercise, since the organism always rested for some seconds before trying it again.

Another type of activity, rarely observed, was a rotating movement on the longitudinal axis, much as a log may roll over and over in the water. This also gains no distance for the parasite, but is continuous and performed rather rapidly, unlike the somersaulting.

Actual travel is accomplished by a method similar to the above, but in this case the organism follows a corkscrew-like path, much as some of the free-living flagellates and ciliates do. Such motion is much more sporadic than with most such protozoa however, and when it is observed, progression seems always to be with the pointed end first.

Usually the rate of movement is relatively slow, and is made in steps with considerable intervals of rest between. However the parasites occasionally travelled across an entire oil immersion field without pausing to recuperate from such strenuous and apparently unaccustomed activity, and could, not infrequently, even be seen moving against the current.

Actual speed was rather difficult to measure, due to the intermittent character of the motion of most of the organisms. Occasionally a crescent could be seen moving as fast as a micron per second, and in one instance a 100-micron-dash was completed in less than 10 seconds. But usually speed was much slower, and it often required a full minute for one of the parasites to cover a distance of 10 micra.

[3] The morphology of *Toxoplasma* is somewhat variable, but when suspended freely, as in exudate, the organism is generally a rather slender crescent, somewhat blunt at one end and moderately pointed at the other, and in cross-section somewhat flattened. Under other circumstances, as in tissue sections, the organisms often look oval or rounded. When free, in our experience, they usually measure from 6 to 10 micra in length and 3 to 4 in width. Perhaps environmental conditions influence their relative size; some observers have reported them to be considerably smaller.

Another type of movement, observed only in the presence of leucocytes or other host cells, was often seen when the parasite came into contact with the cell wall. Not seldom as many as half a dozen organisms could be seen with their (apparently) blunter ends in contact with the surface of a mononuclear cell. Here they would rotate, much as if attempting to bore their way into the cell's interior, the distal end of the crescent often appearing to describe a small arc in the surrounding medium. Considerable effort seemed to be exerted, since the cell was often pushed about by the activity of the parasites.

In one case, actual entrance of a mononuclear cell by a parasite was seen. First, a deep depression appeared in the cell wall as the parasite seemed to push against it, and then, after about 30 seconds, the *Toxoplasma* successfully forced its way in.

Movement of the parasites within cells was also observed. In one such case, one of an intracellular population of 12 organisms was seen to exhibit the typical twisting, corkscrew type of motion within the cell cytoplasm, although the quarters were cramped, with living space of only about 20 micra in any direction.

Actual entry into cells is not often seen, though it has been occasionally reported. Apparently it is a selective process, for although many collisions between parasites and erythrocytes were observed, in no case were the latter penetrated.[4] Or it may be that the physical properties of the mature, mammalian red cell make entry into them difficult, since in numerous cases pressure by parasites under observation was sufficient to heavily indent the cell surface, but the erythrocyte failed to rupture and promptly resumed its shape when the maurauder wandered off.

Still another type of activity was also seen, although it seems to occur only in preparations which have experienced considerable drying, and it may hence be abnormal. In this case, a wave of constriction may often be observed, beginning at the anterior end of the parasite and travelling posteriorly, and followed by others at short intervals. In the meantime the organism may move a very short distance forward, perhaps half its length.

Neither wet nor Leifson-stained specimens showed anything which could be interpreted as flagella, although a cytoplasmic projection at the anterior end could sometimes be seen. This was presumably what Guimarães and Meyer (1942) called a "trombiculum." This organelle, if such it is, was seldom more than one-quarter the length of the parasite. It appeared to be very plastic and retractile, and the crescent often seemed to be using it in tactile fashion, as if it were a probe.

The fact that no flagella were ever seen in living specimens, even with the dark field, or in stained preparations seems to make it very likely that *Toxoplasma* does not possess them. The parasites studied were from many mice, and included a very large number of organisms.

Like most other investigators, we have also seen nothing suggestive of stages in a more complex life-cycle, and it is our impression that the variations in shape

[4] Invasion of avian erythrocytes has however been reported by Wolfson (1941), and by the senior author (Manwell, 1941). Wolfson was dealing with a strain of *Toxoplasma* derived from the guinea pig, and which she had transferred to ducks and canaries. But the strain used in the second study was found in a young English sparrow which had been brought to the laboratory in a moribund condition. Since the infection could not be established in mice, it now seems likely that it was quite different from mammalian *Toxoplasma*.

and size which normally seem to occur are primarily due to differences in parasite environment.

DISCUSSION

It is no longer possible to doubt that *Toxoplasma* is capable of independent movement, although the mechanism of locomotion still remains completely obscure. The fact of locomotor ability is, of course, no evidence that a unicellular organism is a protozoan. Even the demonstration of locomotor organelles may not be enough. But the lack of such organelles and their associated structures, together with the absence of a life-cycle typical of some one group of protozoa, is certainly presumptive evidence that an organism is not a protozoan. So far no one has been able to demonstrate convincingly the possession of either of these things for *Toxoplasma.* Splendore's (1913b) microphotograph of a flagellated form is not clear-cut, nor has any one subsequently been able to confirm his observation of blepharoplasts.[5]

The truth is that *Toxoplasma* does not move as does a typical flagellate, with the rather jerky manner of the latter. Its motion, however, is much like that described by various authors for the spores of *Sarcocystis.* The similarity is so close that it is worth quoting the observations of two of them: Scott (1930) remarked:

> "The Sarcosporidia were nearly all motile, would turn over, straighten out and move one-third the way across the field in a short time; they would twist and turn and change position almost constantly. The hyaline end of a spore would lengthen out, causing the spore as a whole to assume a long spiral shape, then as it contracted and resumed the normal or typical shape the spore moved forward a distance almost equal to its full length. Twisting and turning movements were more characteristic than progressive locomotion."

Darling (1909) also commented on "a very slow, interrupted and gyratory spiral motion" of the spores. Koch (1902) showed a spore in 4 successive positions, as diagrammed below. His figure would apply almost equally well to *Toxoplasma* (Figure 1).

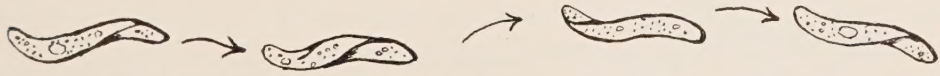


FIG. 1. *Sarcocystis muris,* showing a spore in four successive positions. (Redrawn from Koch.)

Although the striking similarity of the behavior of the "spores"[6] of the two parasites seems very significant, there are numerous other remarkable likenesses and differences. These are summarized in Table 1.

The most significant of these seems to be the almost complete lack of host specificity. *Toxoplasma* has been reported from at least 24 genera of mammals belonging to 7 orders, and from 3 species of birds representing 3 orders.[7] *Sar-*

[5] Laveran remarked, in a note following Splendore's paper, that he and Marullaz had examined "a great number of preparations of *Toxoplasma gondii,*" including some stained with Giemsa, or with iron hematoxylin, but that they were "unable to confirm the existence of centrosomes or flagella."

[6] The term "spore" is unsatisfactory, especially when applied to *Toxoplasma,* but no other seems better to use for the corresponding stages of these two genera.

[7] The numerous host records of *Toxoplasma* in birds, based on the morphology of the parasite alone, are not considered here. There is also uncertainty about many similar reports of infection in mammals.

cocystis has also been identified from many kinds of mammals [according to Kean and Grocott, (1945), 12, belonging to 6 orders], birds (20 species, belonging to 8 orders, 13 families, and 19 genera, were listed by Erickson in 1940), and reptiles (both lizards and snakes).

Although the ability of these parasites to infect so many kinds of hosts has led to the description of numerous species (more than 50 of *Sarcocystis* and at least 24 of *Toxoplasma*) there is general agreement today that but one valid species of *Toxoplasma* exists (*T. gondii* Nicolle and Manceaux, 1909) and it is thought probable that there may also be but one of *Sarcocystis* (this would then be *Sarcocystic miescheriana* (Kuhn, 1865)). This last was the view of Alexieff (1913)

TABLE 1.—Toxoplasma *and* Sarcocystis *compared*

Characteristic:	*Toxoplasma*	*Sarcocystis*
Species:		
Number	probably one only:	probably few, or but one:
Name	*T. gondii* Nicolle & Manceaux 1909	*S. miescheriana* (Kuhn 1865)
Geographic distribution	world-wide	world-wide
Morphology:*		
Vegetative stage (or "spore")		
Shape	crescentic (when free), with a more pointed anterior end; otherwise often ovoid or round	crescentic (when mature), with a more pointed anterior end; otherwise often ovoid or round (said sometimes to be amoeboid)
Size	rather variable (up to about 10 micra long)	highly variable (up to 15 micra long, or even more)
Nucleus	usually nearer blunt end; "loose rosette of chromatin" (Nic. & Manc.)	nearer blunt end; "group of sharply differentiated granules" (Scott)
Locomotor organelles	absent	absent
Other stages	unknown (pseudocysts or "terminal colonies" occur)	Miescher's tubes (spore sacks); elongate, threadlike; up to 20 mm. long
Life-cycle	apparently simple (sexual stages reported, but never confirmed)	may be that of a fungus (*Aspergillus*) (cf. Spindler & Zimmerman, 1945)
Reproduction	longitudinal binary fission only ("spore")	longitudinal binary fission only (spore)
Motility	occasionally rather active; motion intermittent, in spiral path	occasionally rather active: movement pattern like that of *Toxoplasma*
Host range:		
Naturally acquired infection		
Mammals	many species, of numerous orders	many species, of numerous orders but commonest in herbivores and omnivores
Birds	several species, of several orders	numerous species, of numerous orders
Reptiles	lizards and snakes†	lizards and snakes
Invertebrates	unknown	unknown
Experimental susceptibility		
Warm-blooded animals	very wide (but behavior of parasite varies with strain, and type of host)	wide (but morphology of parasite and character of infection vary with host type)
Cold-blooded animals	frogs and snakes attempted, without success	no data
Invertebrates	parasite survives a few hours, or even a few days in some arthropod hosts (e.g. *Pediculus humanis*)	no data
Tissue susceptibility	reticulo-endothelial system preferred; occurs commonly also in brain, liver spleen, muscle, etc.	skeletal muscle, or myocardium
Pathogenicity	not usually highly pathogenic (no toxin known)	not usually very pathogenic (but produces toxin: "sarcocystin")
Most susceptible laboratory animal	mouse (highly fatal)	mouse (highly fatal)
Sabin-Feldman dye test for infection	positive	positive

* Some differences in accounts by different authors.
† Not, however, substantiated by successful experimental transfer to mammals.

and Wang (1950). Scott (1930), though he did not go quite as far as to claim but one species of *Sarcocystis*, conceded that nearly all described species in the genus were invalid.

In any comparison of *Toxoplasma* and *Sarcocystis* not only the morphology (see Table 1) and the behavior of the vegetative stage ("spore") must be considered, but also the nature of what has been called the "pseudocyst" of *Toxoplasma*, and of "Miescher's tubes" in the case of *Sarcocystis*. Although these seem very different, it appears likely that their origin, in each case, is largely or entirely from the host's cells or tissues. There is little evidence of local tissue reaction to either parasite.

Especially significant, perhaps, is the mode of reproduction, which is in both parasites longitudinal binary fission. Both also show a marked antero-posterior differentiation. Considered by themselves, these characteristics might seem to point toward relationship to the flagellates, but in the absence of flagella and accessory structures, such as blepharoplasts and parabasal bodies, they seem only to be further evidence of basic similarities between the two genera.

Another fact, often overlooked in considering the problem of whether either of these two genera should be classed as SPOROZOA, is their apparent lack of any intermediate host or vector. The great variety of host species appears to make the existence of any invertebrate vector at least highly unlikely.

Whatever the final decision of the taxonomist may be, it seems at least very probable that *Sarcocystis* and *Toxoplasma* are rather closely related. Similar serology is often indicative of biological kinship, and Muhlpfordt (1951), as already mentioned, has recently shown that both toxoplasmosis and sarcosporidiosis react similarly to the Sabin-Feldman dye test. This is a highly significant fact, if confirmed.

The problem remains as to the essential nature of *Toxoplasma* and *Sarcocystis*. It has been long recognized that neither fits well into the scheme of protozoan classification. Spindler and Zimmerman (1945) were apparently able to show that *Sarcocystis* would develop typical fungal stages in sterile dextrose culture solution, and that young pigs, when fed such stages, exhibited typical sarcocysts several months afterwards. This suggests that *Sarcocystis*, and hence perhaps *Toxoplasma*, is not a protozoan, though it needs confirmation. The remarkable host-range of both organisms certainly points in the same direction.

It is true that attempts to grow *Toxoplasma* on Sabouraud's medium (widely used for fungi) have failed, and we also have been unsuccessful in growing it on dextrose solution, as Spindler and Zimmerman did *Sarcocystis*. It also reacts differently to Feulgen's stain than do the yeasts (Cross, 1947), but neither of these facts is sufficient evidence for calling it a protozoan.

SUMMARY

A thorough study of *Toxoplasma* in peritoneal exudate and in physiological saline, using both bright and dark-field illumination, has confirmed the fact of its motility, and has shown that it exhibits several different types of behavior. There was no evidence of flagella or the usual accessory structures seen in flagellate protozoa, either in living or stained organisms. Behavior was strikingly like that of *Sarcocystis* spores. This fact, with other evidence, points strongly to a close biological

relationship of the two genera. There seems much reason to question the general practice of including either with the protozoa.

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THE EFFECT OF FREEZING ON ENCYSTED *ANISAKIS* LARVAE[1]

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Immature heterocheilid nematodes, usually *Contracaecum* or *Anisakis,* are commonly found encysted in commercial fish brought to the Seattle docks. Sites of predilection are the serosal surfaces of the stomach, pyloric caeca, and adjacent intestine, the ventral mesentery, and less commonly, somatic musculature in contact with these areas or along the vertebrae. The heaviest parasite load occurs in bottom fish, such as rock fish, cod, and flat fish, which may carry several hundred parasites per host. Herring and salmonids are usually more lightly infected.

Criteria for specific identification of the immature nematodes in this group are not established. *Anisakis* adults have been reported as occurring in various marine mammals (pinnipeds and cetaceans), and occasionally from marine birds. Recently, immature stages were reported from man (Hitchcock, 1950). There is nothing to suggest that more than one species of *Anisakis* is present in the material from the intermediate hosts encountered in Seattle. Adult *Contracaecum* occur locally in marine mammals, birds, and fish (*Sebastodes,* cod, etc.). Since a temperature of 37° C. is lethal *in vitro* for most excysted *Contracaecum* from fish in this area, it seems likely that the common final host is a piscivorous fish.

No published material dealing specifically with death by freezing has been found for this group. The only example of encysted nematode larva investigated with respect to freezing is that of *Trichinella spiralis,* which has been the subject of repeated testing to establish a basis for control of trichinosis. This literature has been most recently reviewed by Gould and Kaasa (1949). Discrepancies among the opinions expressed have been attributed to the type of freezer employed, the medium surrounding the sample, the size of the sample, the rate of cooling, the age of the larvae, individual differences in the larvae, and the host in which they are encysted. It seems probable from this work that trichina larvae may survive for two weeks or more at – 10° C., less than 3 days at – 20° C., and only a few hours at – 30° C.

Studies to determine the effect of freezing on heterocheilids were initiated following inquiries from the Woodland Park Zoo, Seattle, where frozen herring are fed to seal, cormorants, and other potential hosts. Interest has also been expressed in this problem by animal breeders using fish scrap, and by individuals with public health responsibilities.

MATERIALS AND METHODS

For freezing experiments under controlled conditions, encysted *Anisakis* were removed from *Sebastodes* scrap at Seattle processing houses. Uninfected tissue was trimmed off, leaving high concentrations of parasites. The parasitized tissue

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was pooled and divided into 120 gram amounts. One of these samples was digested in a pepsin-HCl bath at 37° C. as a control to determine the number of naturally occurring dead larvae; material was not used if the non-viable larvae exceeded 5%. The other samples were wrapped in aluminum foil with a low temperature thermometer inserted to the center of the mass. After precooling for a few hours at +5° C., these packages were laid on metal shelves in various freezing chambers; temperatures of the chambers and of the samples were recorded at frequent intervals.

The samples were exposed to temperatures of −5°, −10°, −17°, −30°, and −42° C. A separate freezing compartment was used for each temperature. Experiments were disqualified if the chamber temperatures, read at close intervals, varied by more than one degree. The temperature rcorded for the sample was that of the central portion of the mass, and did not take into account the gradient including the outer, colder portion.

The packages were removed at predetermined intervals, allowed to thaw at room temperature, and digested in a pepsin-HCl bath at 37°. The criterion used for viability was movement, however feeble, either spontaneous or in response to mechanical or light stimuli. Hosts which would allow a normal and predictable infection were not available. Total larvae counts of 200 or less are exact; under 400, accurate to the nearest 5; over 400, to the nearest 10.

Additional experiments with smaller numbers of larvae were performed using intact herring, and with packages or test tubes immersed in methyl cellusolve at a constant temperature; these made possible limited exploration of variants such as the rate of cooling and influence of immersing fluids.

COMMERCIAL FREEZING

At the cold storage warehouse used by the local zoo, herring taken in the spring run are frozen in 100 lb. blocks. The blocks are first exposed to −20° to −30° F. (−28.8° C. to −33.4° C.) for about 16 hours and then stored at about +10° F. (−12.2° C.) until used. A sample of 5 herring in one lot taken at the end of the first 24 hours of this sequence revealed 61 *Anisakis* larvae and more than 500 *Contracaecum* larvae, all of which were dead. A larger sample from a second lot with identical treatment, in which a 25 pound lot containing 86 herring was examined, disclosed over 3000 *Contracaecum,* all dead, and 320 *Anisakis* of which 41 were alive and apparently unharmed. In several other samples taken after intervals of one week to several months of storage, several hundred *Anisakis* were encountered, all of which were dead.

Anisakis larvae appear to be more resistant to cold than *Contracaecum;* this was substantiated by preliminary laboratory experiments involving both genera. As a result *Anisakis* was chosen as a better indicator to determine the minimal lethal freezing interval.

RESULTS AND DISCUSSION

The results from exposure to specific temperatures are listed in Table 1.

With the moderate temperatures used in Series A (−5° C.) and in Series B–D (−10° C.), most of the larvae were killed within 72 to 96 hours. However, survivors appeared in nearly every sample; in Series D, 38 of 1029 larvae were still

alive and apparently unharmed after 12 days. When the cysts were cooled to −17° C., no survivors were encountered after 24 hours in the standard experimental packages. Intact herring (Series F), exposed to this same temperature, contained

TABLE 1

Series	Chamber temp.	Internal temp. of sample when removed	Time exposed to cold chamber	Total *Anisakis*	*Anisakis* alive	Approx. % alive
A	−5° C.	−5° C.	2⅔ hours	520	400	77
		"	20 "	46	16	35
		"	96 "	364	20	5.5
		"	144 "	163	10	6
B	−10	−10	24 "	207	10	5
		"	48 "	250	4	2
		"	72 "	430	6	1
C	−10	−10	3 days	525	3	.6
		"	4 "	540	3	.5
		"	5 "	825	29	3.5
		"	7 "	675	10	1.5
		"	8 "	540	0	0
		"	9 "	675	3	.4
		"	10 "	465	1	.2
		"	11 "	740	0	0
D	−10	−10	8 "	870	4	.5
		"	9 "	580	1	.2
		"	10 "	880	10	1
		"	11 "	640	2	.3
		"	12 "	1029	38	4
E	−17	−14	6 hours	600	33	5.5
		−17	24 "	710	0	0
		"	30 "	550	0	0
		"	75 "	590	0	0
F*	−17	−14	5 "	39	2	5
		−11	10 "	49	28	57
		−17	15 "	65	0	0
		"	20 "	94	0	0
		"	25 "	53	0	0
		"	30 "	85	0	0
G**	−20	−20	1½ "	325	0	0
H**	−30	−30	1½ "	270	0	0
I	−42	−42	18 "	600+	0	0
J	−42	−21	2 "	850+	15	2
		−20	2 "	157	0	0
		−23	2 "	500+	0	0
		−27	2 "	900+	0	0
		−30	2¼ "	600+	0	0
		−30	2½ "	900+	0	0
		−36	3 "	750+	0	0
		−42	3½ "	850+	0	0
		"	4 "	750+	0	0
		"	4½ "	900+	0	0
		"	5 "	750+	0	0
		"	5½ "	800+	0	0
K**	−30					
(1)		−30	5 min.	45	0	0
(2)		−5	½ "	12	12	100
		−10	1 "	12	11	92
		−17	1½ "	12	3	25
		−20	2 "	12	0	0
		−21	2½ "	12	0	0
(3)		−4	½ "	12	12	100
		−10	1 "	12	10	83
		−17	1½ "	12	2	17
		−20	2 "	12	0	0
(4)		−5	½ "	12	12	100
		−10	1 "	12	10	83
		−17	1½ "	12	0	0
		−20	2 "	12	0	0

* Intact herring used.
** Glass or aluminum vessels immersed in bath of methyl cellusolve–CO_2 ice.
K(1) cysts in fish tissue.
(2) cysts suspended in mammalian Ringer's solution.
(3) cysts suspended in proteose-peptone broth.
(4) cysts suspended in beef serum.
All control samples showed at least 95% viable larvae.

no live *Anisakis* after 15 hours; however, the number of larvae recovered in this series would not be sufficient to demonstrate small percentages which might be expected to survive at a critical period. At the lower temperatures (−20° to

– 42° C., series G–J), all *Anisakis* are rapidly killed; no survivors remained beyond 2 hours at – 21° (Series J).

Anisakis larvae thus appear to be more sensitive to freezing than *Trichinella* at all of the temperatures tested. As with prophylactic freezing of pork, the size of the mass must be considered; the rate of cooling of 100 lb. blocks of herring apparently does not allow the central part to become cold enough in 16 hours in a chamber at about – 30° C. to kill all *Anisakis*. If time is allowed for all parts of the fish to cool to the chamber temperature, freezing in home or commercial freezers at – 17° to – 30° C. for 24 hours is adequate to kill the encysted heterocheilids.

The rate of cooling (Series G to J compared with K) appeared to exert no influence on the rate of killing, nor did the medium surrounding the cysts (Series K). Irregularity in the rate of cooling in separate samples in the same freezing chamber is noticed in Series F and J. Individual differences between larvae presumably account for the occasional survivors in marginal treatment.

No measure of the infectivity of the surviving *Anisakis* larvae was available. Experimental infection of mammals or birds with this genus has not been reported. Our attempts to establish infections in kittens and puppies by feeding normal cysts have failed. Reduced infectivity after exposure to freezing has been reported for *Trichinella* without obvious alteration of the motility and general appearance of the larvae (Ransom, 1916; Augustine, 1933). It seems likely that a similar reduction in infectivity would occur among the *Anisakis* survivors, many of which show signs of impaired movement.

Although associated with gastric ulcers in pinnipeds, *Anisakis* has not been shown to be a causative agent for this or other pathological condition. The esthetic implications are undoubtedly the major consideration concerning fish containing *Anisakis* as food for man.

SUMMARY

Immature encysted *Anisakis* sp. from commercial fish from Puget Sound were exposed to various freezing temperatures to determine resistance to cold. At – 5° and – 10° C., most of the nematodes died in the first 24 hours; the few remaining survivors may remain alive for several days. At – 17° C., all died within 24 hours. At – 30° C. all were killed in five minutes.

Commercial freezing of herring in 100 lb. blocks using initial temperature of about – 30° C. for 16 hours and storage at – 12° will kill any of the *Contracaecum* larvae and nearly all of the *Anisakis* larvae in the first 24 hours. The few survivors apparently die within the first week.

Death was interpreted as absence of movement, spontaneous or induced. Determination of viability of surviving worms by infecting laboratory hosts was not accomplished.

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VIABILITY STUDIES ON EGGS AND INFECTIVE LARVAE OF *NEMATODIRUS SPATHIGER* (RAILLIET, 1896), A TRICHOSTRONGYLID NEMATODE OF RUMINANTS[1]

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The experimental results of Kates and Turner (1953) showed that heavy infections of *Nematodirus spathiger* can retard growth and produce diarrhea and emaciation in lambs. These authors also summarized case reports in the literature, in which natural infections of this parasite or other species of the genus were considered the principal or secondary cause of various clinical symptoms and losses. The pathogenicity of *Nematodirus* spp. to sheep is now reasonably well established. Effective control measures against these parasites must be based in part on reliable information on the survival of their free-living stages under exposure to varying climatic conditions throughout the year in different geographical areas. Therefore, the work reported herein was undertaken to determine the degree of resistance of the infective larvae of *N. spathiger* to the usual year-round climatic conditions of Maryland and to artificial conditions in the laboratory, as well as to determine the viability of eggs after drying.

SUMMARY OF LITERATURE

Some information is available in the literature on the effects of various pasture- and laboratory-imposed conditions on the longevity of the free-living stages of *N. spathiger* and of two closely related species, *N. filicollis* and *N. helvetianus*. In the pasture reports, the survival of the preparasitic stages of these parasites was usually determined by grazing at various times of the year parasite-free lambs at intervals on pastures contaminated with eggs of the worms in question. In the laboratory studies, the larvae were subjected to various artificially imposed conditions and examined for normal appearance and motility.

Pasture observations. Dikmans and Andrews (1933) and Kates (1943, 1950) in Maryland, Hawkins et al (1944) in Michigan, Griffiths (1937) and Swales (1940) in Canada, and Taylor (1934) in England reported that some preparasitic stages of *Nematodirus* spp. survived the winter months on pasture, whereas, Zawodowsky and Zviaguintzev (1933) in Russia reported that larvae of *N. helvetianus* did not survive exposure to winter weather in the region of Moscow. Shorb (1942) noted that *Nematodirus* spp. failed to develop in any appreciable numbers in July on a Maryland pasture, but the numbers of *Nematodirus* on pasture may have been insufficient to cause infection in the experimental animals. Kates (1950) observed some survival of *Nematodirus* spp. through the summer months. Doll and Hull (1946), however in Kentucky, reported *N. filicollis* to be the only species in several genera tested to survive during the summer and fall months.

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[1] This paper is part of a thesis submitted to the Graduate School, University of Maryland in partial fulfillment for the Master of Science degree.

Laboratory observations. Boulenger (1915) observed that larvae of *N. filicollis* withstood long periods of drying and survived repeated drying and remoistening 12 times in 6 days. However, the maximum period the larvae survived complete desiccation was not determined, whereas ensheathed larvae survived 11 months in water. Zviaguintzev (1934) reported that larvae of *N. helvetianus* were very resistant to drying, some surviving 8 months in this condition at temperatures of 64° F. to 72° F. At temperatures of 42° F. to 48° F. these larvae had a higher

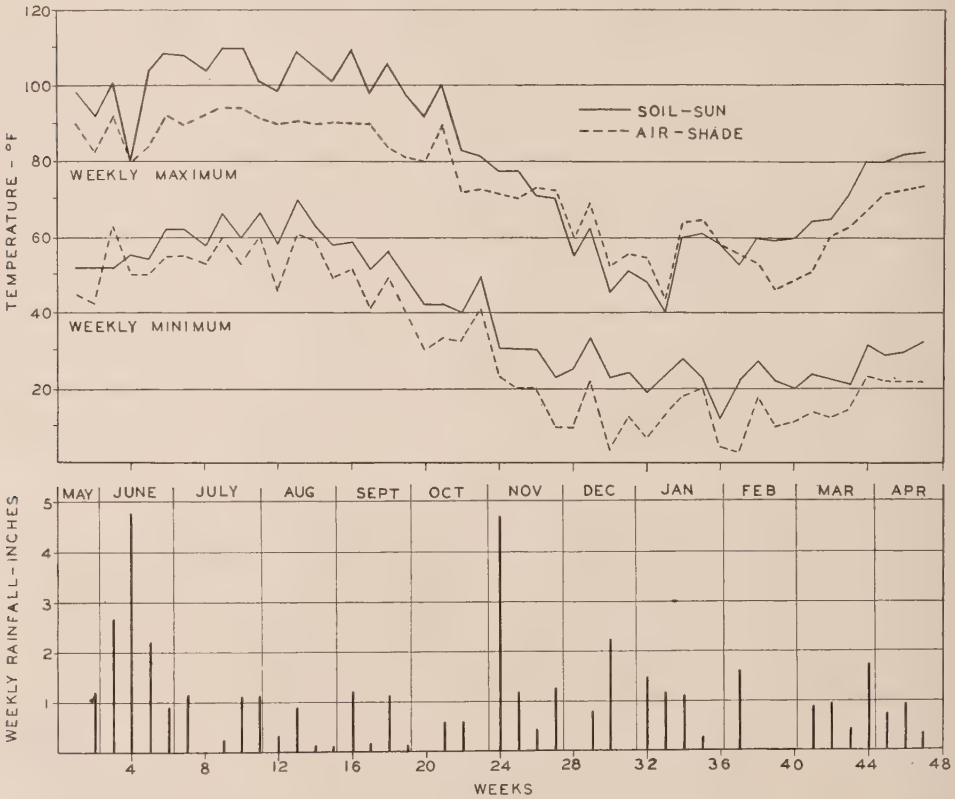


Fig. 1. Temperature and rainfall data for period covered in pasture experiment.

survival rate in water for a greater length of time (up to 5 months) than those exposed after desiccation.

MATERIALS AND METHODS

Pasture experiments. Infective (third stage) larvae used in these experiments were obtained from cultures of sheep feces containing only eggs of *N. spathiger.* Twelve experimental plots were used, upon which viable, infective larvae were exposed to natural weather conditions. Larvae were placed on six plots on May 15, 1951 to obtain data on the larval survival rate during the late spring, summer, and fall, and on six additional plots on November 3, 1951 to obtain comparable data during the winter and early spring. These plots consisted of uncontaminated areas of unshaded grass enclosed by galvanized metal cylinders, each 6 inches in diameter. These were placed in the ground to a depth of 2 inches, with 3 inches remaining

above the surface. These plots were arranged in three rows of four each equidistant apart, in an area approximately 4 feet by 3 feet. Approximately 5,000 infective larvae in water were scattered over the surface of each plot by pipette. The pasture grasses were mainly rye, Kentucky blue, and red top species. The grass in the plots was clipped periodically to maintain its height at the 3 inch level, but not removed. This pasture had not been grazed by sheep for at least two years. The experimental plots were surrounded by a heavy wire fence to prevent extraneous contamination, and were well drained.

At approximately 30-day intervals after larval contamination of the plots, one plot for each interval was sampled for living larvae as follows: larvae were recovered from each plot sampled by Baermannizing and washing separately, as described in the next section, a) the grass (all the grass clipped therefrom), b) the humus (the remaining grass stubs, plus one-half inch of soil), and c) the soil (sub-surface down to 2 inches). The plots were removed from the pasture between 9:00 and 10:00 A.M. before the occurrence of that day's maximum temperature. Only living larvae were counted. A few obviously dead larvae were recovered from all plots sampled, but these were not included in the counts.

Efficiency of Baermann apparatus. The apparatus described by Baermann (1917) was employed in recovering nematode larvae from the soil plots, and its efficiency for recovery of *N. spathiger* larvae was tested prior to its use in the survival experiments. Sandy and clay loam soils and grass, as encountered on the pasture, were placed in screens and put into Baermann funnels, after water had been added to a point just below the screen level in the funnels. Then 5,000 larvae were counted by standard dilution technique (variability not exceeding 5%) and gently pipetted evenly over the surface of the material on each screen and mixed carefully with it. Water was then added from the lip of the funnel to bring the water level to the surface of each test material. The percentage of the larvae recoverable from the different materials by the Baermann apparatus was determined by collecting after 24 hours 50 ml. of water from each funnel and examining it for larvae. Of the 5,000 *N. spathiger* larvae mixed with the various test materials, a 41.8 per cent return was obtained from grass, 15.7 per cent from clay loam and 13.4 per cent from sandy loam. On the basis of these findings an attempt was made to modify the method to obtain a greater per cent of larvae. This was achieved to some extent by washing the test materials through the screens into separate beakers after Baermannization with a stream of water and collecting the washings. A uniform number of sedimentations and decantings were done on these washings, and the living larvae in the remaining sediment were counted. These counts were added to the larval counts obtained by the standard Baermann technique to give the final survival figures shown in figure 2.

Laboratory experiments. To determine the effect of desiccation on infective larvae, approximately 100 larvae were placed on each of 26 separate slides and allowed to dry at room temperature, drying being completed in about 6 hours. The slides were kept under laboratory conditions at temperatures ranging from 70° F. to 90° F. One slide was examined at each successive bi-weekly interval until the series was completed. Water was added to the slides to revive those larvae still alive, and the percentage of larval survival determined as before.

The exposure of the larvae to continuous freezing was done as follows: 100

larvae were pipetted with 5 ml. of water into each of 6 small vials; these were exposed to subfreezing temperatures varying between 28° F. to 30° F. At bi-weekly intervals a vial was removed from the refrigerator, thawed at room temperature, and its contained larvae examined for motility. The larvae in these vials were thus examined over a period of three months, which would be more than the maximum period in this area to expect prolonged freezing. These same larvae, thereafter, were retained in the refrigerator at the same temperatures and were re-examined at varying periods up to 10 months to determine the effect of further freezing.

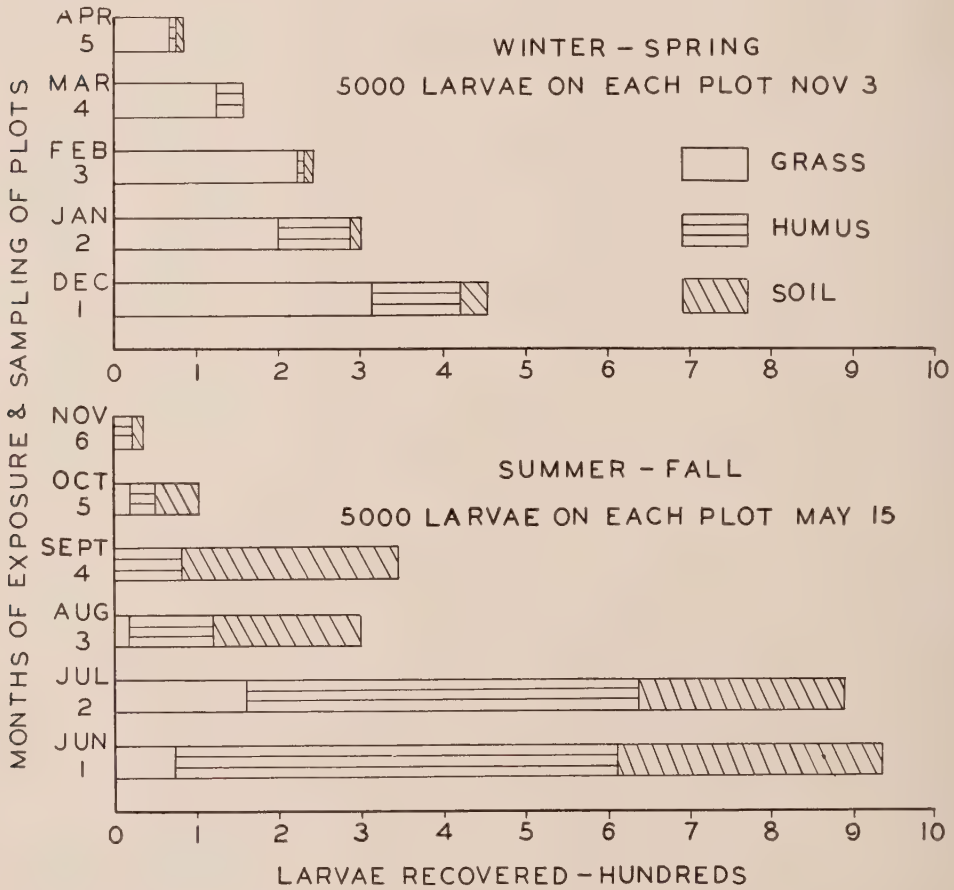


FIG. 2. Recovery of living, third stage larvae of *Nematodirus spathiger* from experimental grass plots.

The effect of alternate freezing and thawing was also tested. Approximately 100 larvae were frozen as before for a continuous period of approximately 4½ months, and then thawed, examined, and refrozen at the following intervals: bi-weekly intervals for about 1½ additional months, weekly for 1 month, monthly for 3 additional months and terminally 6 times within 3 days. Total elapsed time was about 10 months. The reason for these changes in testing intervals was that it soon became apparent that survival was relatively high after such exposures. A vial containing 100 larvae in water was also kept at approximately 35° F. for 11 months and tested each month for larval survival.

In conjunction with the tests mentioned above on infective larvae, the effect of drying upon the eggs of *N. spathiger* was also studied, samples of desiccated eggs being maintained at room temperature (70° F. to 80° F.) and 40° to 42° F. Slides upon which the eggs were dried, were examined monthly, after being moistened, for larval development.

CLIMATOLOGY

Weather data was obtained in the vicinity of the experimental pasture plots for the test period: May 15, 1951 to April 15, 1952. Air temperatures were taken in shade and soil temperatures taken by placing the thermometer just below the soil surface, immediately in front of the first row of plots to simulate those temperatures within the cylinders. Temperatures were not taken within the cylinders as it was not desirable that the plot material, containing the applied larvae, be disturbed. Data on the daily precipitation and daily maximum and minimum air and soil temperatures were recorded. These are summarized on a weekly basis in figure 1. Over the winter snowfalls were infrequent and snow did not remain on pasture for more than a week. Frequent freezing and thawing occurred, which is characteristic of this region.

RESULTS

Pasture Experiments

For convenience the data of the pasture experiments are divided into two periods and are summarized in figure 2.

Summer-Fall Period. The highest recovery of 5,000 larvae exposed May 15 was obtained from the plot tested on June 15, 923 or 18.4 percent of the total exposed, and the lowest recovery on November 15, 30 or 0.6 percent of the total exposed. The highest percentage of larvae was recovered from the humus sample during the comparatively wet, warm, periods of June and July, when the air temperature reached a maximum of 94° F. and the soil temperature 108° F. During the entire summer-fall period 90 percent of living larvae were found in the humus and in the soil. The first exsheathed larvae were recovered from the September plot, and some of those recovered in November were vacuolated and exsheathed. Many larvae had short tail sheaths, the greater portion having been broken off and lost. The larvae after exposure to pasture conditions for 2 months exhibited decreased motility, and after 6 months became very sluggish.

Winter-Spring Period. The majority (70 percent) of the larvae were on the grass in this group. The greatest number of larvae, 462, were obtained from the December plot, the least number, 70, from the April 15 plot. Throughout the winter more larvae were on the grass than in the soil. Unlike the debilitated larvae recovered after 4 months on pasture during the summer, those recovered after a similar period during the winter were ensheathed and in excellent condition. However, the total recovery of larvae from these plots was smaller than experienced in the summer-fall experiments. This may not have been due to the low winter temperatures, but to inherent variability in the technique used in recovery of the larvae.

Laboratory Experiments

Desiccation. When viable larvae were obtained from culture and dried on slides in the laboratory they became tightly coiled, wrinkled, brittle to the touch, and their internal structures appeared distorted. Upon the addition of water to these prepara-

tions to determine the percentage of larval survival, those still alive absorbed water and assumed their normal shapes and movement. Some larvae, although ap-

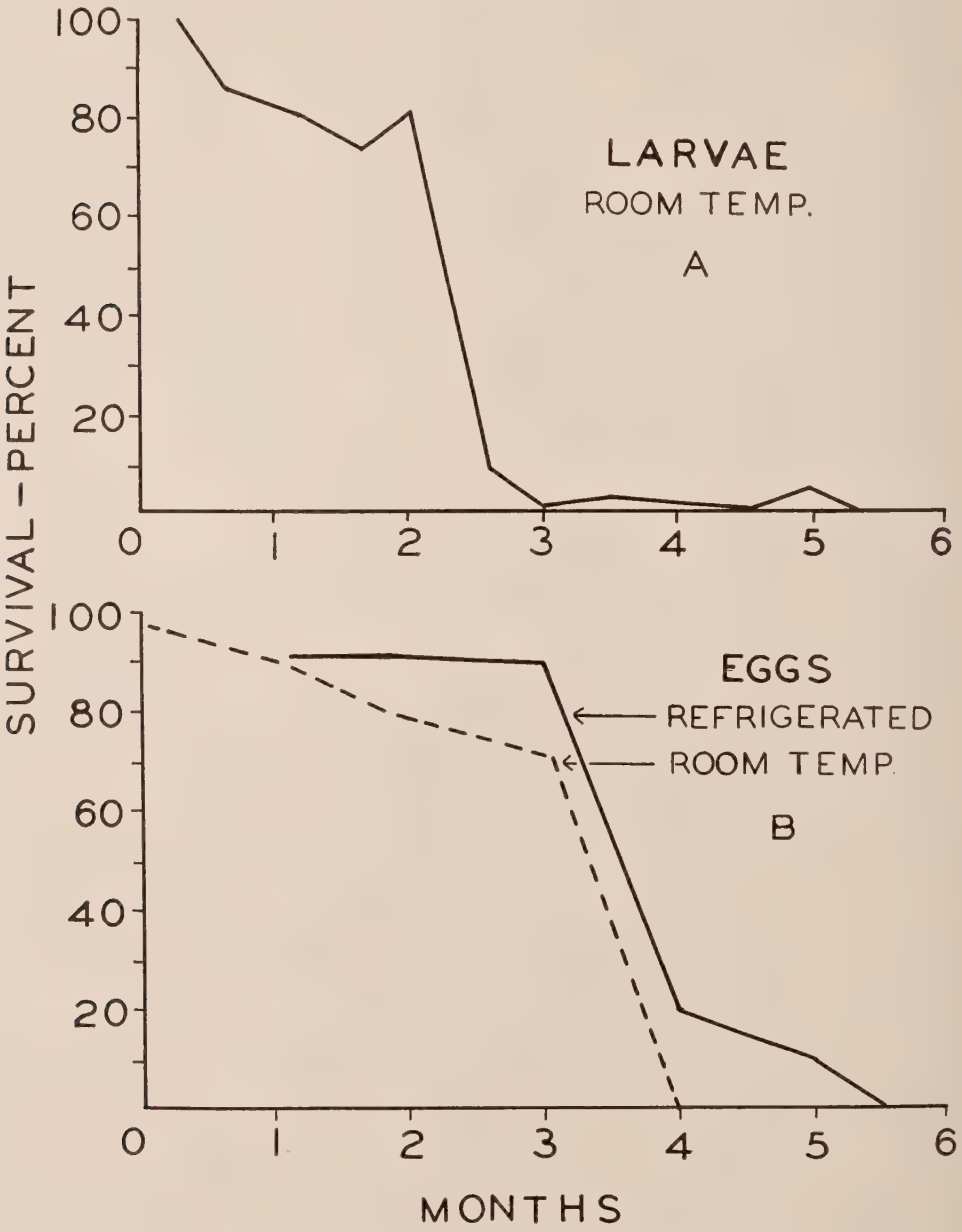


FIG. 3. Effect of desiccation upon larvae and eggs of *N. spathiger*. A. Desiccation of larvae at temperatures of 70°F. to 90°F. B. Desiccation of eggs at temperatures of 70°F. to 80°F., and at 40°F. to 42°F.

parently absorbing water, uncoiled into the characteristic "C" shape of dead larvae. This free absorption of water displays the permeability of the larval sheath to moisture.

As shown in figure 3A, dried, infective larvae survived very well exposure to room temperatures for about 2 months, 82 percent surviving. In subsequent viability tests only a small percentage or none were alive; 7.9 percent were alive after approximately 2½ months of drying, none at the 3rd month, a few after 3½ months, none at 4½ months, and 4.6 percent alive on the 5th month, all being dead thereafter. After the second month of exposure the recovery of the larvae when moistened was delayed, and their movements sluggish, some showing motility only when touched with a dissecting needle. Prior to this study the writer dried some larvae and kept them at a temperature of 40° F. When these were tested for motility at 6 and 11 month intervals thereafter, 6 percent and 1 percent respectively, were still alive.

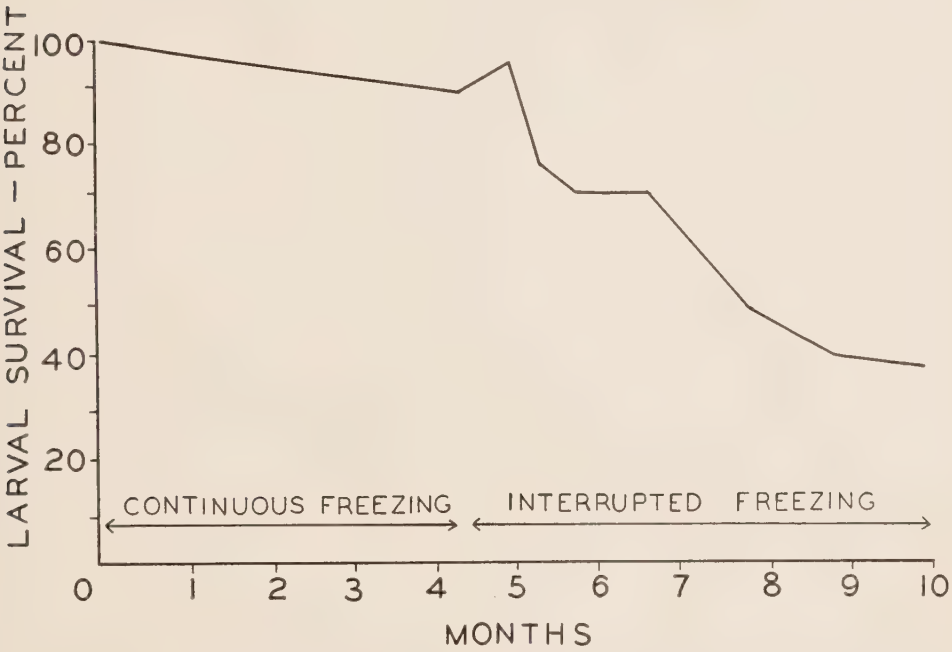


Fig. 4. Effect of continuous and interrupted freezing upon *N. spathiger* larvae.

The resistance to desiccation of partially embryonated eggs was also studied to compare the hardiness of the egg and larva, (fig. 3B). The dried eggs kept at 40° F. to 42° F. had a higher survival rate than those kept at room temperature. None kept at room temperature survived 4 months exposure, whereas 10 to 20 percent of the eggs kept at 40° F. still survived up to 5 months.

Freezing. Larvae continuously frozen in water at 28° F. to 30° F. survived remarkably well. After 3 months exposure 98.7 percent were found to be very active when thawed and brought to room temperature and exhibited no apparent adverse effects. The percentage survival from the first examination at 2 weeks to the terminal one at 3 months did not fall below 94, and after a more extended freezing period of 10 months, 25% still survived. The larvae also proved to be similarly resistant to repeated freezing and thawing, (fig. 4); when frozen for approximately 5 months 96 percent survived, when thawed and examined at biweekly intervals for about a month thereafter 72 percent survived, and after re-

peated thawing and refreezing at intervals thereafter 37 percent survived when the test was terminated at about 10 months. Repeated thawings thereafter, 6 times in 3 days, did not alter this percentage.

Over 90 percent of larvae in water at 35° F. survived 8 months, 88.4 percent 9 months, and 48 percent 11 months.

DISCUSSION

Pasture Experiments. By use of the Baermann apparatus and subsequent washing and sedimentation of the grass, humus, and soil samples from the experimental plots, an effort was made to recover and count all viable larvae; however, it is unlikely that a 100 percent recovery was attained. With this in mind the results of tests over the summer-fall period show that at least 7 percent of the larvae exposed survived the entire summer period (May 15 to September 14), and 0.6 percent lived through the summer and fall months (May 15 to November 15). The results for the winter-spring period show that at least 4 percent of the exposed larvae survived from November 3 to March 5, and at least 2 percent to April 15. In both tests of larval survival over the summer-fall and winter-spring periods, the maximum recovery of larvae was obtained at the end of the first month after exposure on the plots, and thereafter the total larvae recovered from the plots in succeeding months gradually declined, with one slight exception, the September test in the summer-fall group. This work confirms and extends the observations of other workers, already cited in this paper, who generally employed parasite-free lambs to determine the presence of viable larvae on pasture, that infective larvae of *Nematodirus* spp. survived better on exposure to climatic conditions throughout the year than the larvae of most other common nematode parasites of sheep. It is noted that the survival results for the winter period reported herein are in agreement with those of most other workers, except for those of Zawodowsky and Zviaguintzev (1933), who reported that *N. helvetianus* larvae did not survive the winter in the vicinity of Moscow.

Laboratory experiments. Zviaguintzev (1934) reported that desiccated *N. helvetianus* larvae survived 8 months at temperatures of 64° F. to 72° F., whereas in this report a small percentage of *N. spathiger* larvae survived desiccation only 5 months at temperatures of 72° F. to 90° F.

Boulenger (1915) also observed that *N. filicollis* larvae could survive desiccation "for a very long time." The higher temperatures at which *N. spathiger* was exposed in the writer's experiment was probably an important factor in their shorter longevity as compared to *N. helvetianus* in Zviaguintzev's (1934) study. However, these results coupled with the excellent survival of the larvae in a warm-moist environment on pasture, show these forms are very resistant to any desiccation that climatic conditions might impose. Drying at low temperatures seems to be less harmful to the larvae than when dried at high temperatures. This may be due to the fact that reasonably complete desiccation is difficult to attain or maintain at low temperatures.

Fallis (1938) observed that eggs of this species were normal after exposure to –10° C. (14° F.) for a two-week period. It was noted by the present author that a portion taken from a mass of fecal material, and allowed to remain at a temperature of 38° F. to 40° F. for 2 years, yielded some living *N. spathiger* larvae upon culture.

Boulenger (1915) found that some *N. filicollis* eggs dried in feces for 20 months hatched when cultured. In the present study partially embryonated eggs of *N. spathiger* had slightly greater resistance to desiccation at room temperature than the hatched larvae. It is reasonable to assume that the thick egg membranes are added protection for the developing larva contained within. Although the desiccated eggs showed a slightly higher survival rate than the desiccated infective larvae for the first 3 months, both were about equally susceptible to desiccation after this period.

As shown by results of the continuous and interrupted freezing tests, the larvae of *N. spathiger* should experience little difficulty in surviving the normal winter weather in Maryland since winters in this state are comparatively mild and accompanied by much alternate freezing and thawing. This is confirmed by the aforementioned winter pasture results.

Infective larvae of *N. spathiger* are especially adapted to endure the annual variations of Maryland weather on pasture for extended periods, and, according to the literature, this is also true for many other areas of the world.

SUMMARY

1. Third stage infective larvae of *N. spathiger* possess unusual ability to survive on pasture under the annual complex of weather conditions of Maryland.

2. Although in general more living larvae were recovered from the test plots during the summer and fall than during the winter and spring, many of those recovered during the former period were in poor condition and exsheathed, whereas all those recovered during the latter period were sheathed and in excellent condition.

3. In the winter-spring period 70 percent of the larvae recovered were from grass, whereas in the summer-fall period 90 percent of those recovered were from the humus and sub-surface soil.

4. Desiccation imposed the greatest hardship on the larvae. After drying 2 months at room temperature, 82 percent survived, but none survived after 5 months. Those dried and maintained at 40° F. to 42° F. had a survival rate of 6 percent at 6 months and 1 percent after 12 months.

5. Survival of partially embryonated eggs dried at room temperature was 70 percent after 3 months with negative results at 4 months, whereas the survival rate of eggs dried at 40° F. was 85 percent at 3 months, 20 percent at 4 months, 10 percent at 5 months, and negative about 6 months. Some survival of eggs was noted after a 2-year exposure in fecal material kept at 40° F.

6. Freezing temperatures of 28° F. to 30° F. had relatively little effect on the larvae. After 3 months 98.7 percent survived, and 25 percent were alive after 10 months exposure. Larvae subjected to alternate freezing and thawing had a survival rate of 72 percent after 7 months, and 37 percent after 10 months exposure. Larvae maintained at 35° F. for 11 months in water had a survival rate of 48 percent.

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HYMENOLEPIS PARVISSIMA N. SP., A MINUTE CESTODE FROM THE SHREW *SOREX BENDIREI BENDIREI* (MERRIAM) IN CALIFORNIA

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University of California, Los Angeles

About one hundred cestodes were recovered from the small intestine of a shrew collected August 11, 1949, seven miles east of Bayside, Humboldt County, California. The cestodes were fixed in Bouin's fluid and stained with Grenacher's borax carmine, or a very dilute aqueous solution of Ehrlich's haematoxylin.

Since these specimens cannot be identified as any of the known species, they are herein described as new and the name *Hymenolepis parvissima* is proposed for them. The following diagnosis is based on the study of thirty specimens. Unless otherwise stated all measurements are given in millimeters.

Hymenolepis parvissima n. sp.

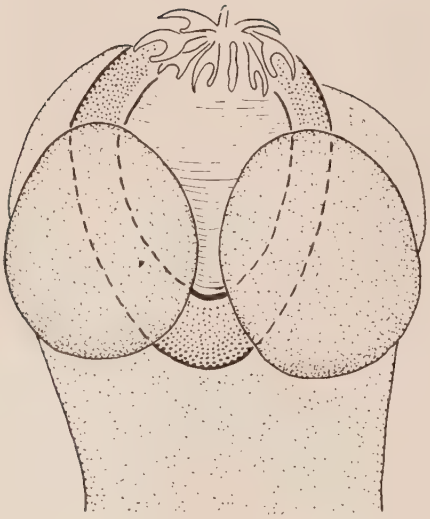
Diagnosis: Strobila total length 0.467–0.850 in mature specimens, average 0.644; strobila greatest width 0.153–0.204; proglottid number 7–10, most frequent number 8. Scolex with well developed rostellum bearing 10 hooks. Scolex diameter range 0.090–0.150, average 0.109; sucker diameter range 40–60 × 48–74 μ, average 51 × 60 μ; rostellum diameter range 50–72 × 60–90 μ, average 61 × 75 μ; hook length 20–24 μ. Genital pores unilateral, situated in anterior fourth of lateral margin of segment. Testes in a straight line, diameter range 20–40 × 28–48 μ, average 28 × 35 μ. Cirrus spinose; cirrus pouch length 60–86 μ. Vagina with seminal receptacle about as wide as cirrus pouch. Ovary bipartite, situated in anterior half of proglottid. Uterus sac-like. Eggs mostly spherical (30–40 μ in diameter), occasionally ovoid (30 × 44 μ) in outline; onchosphere diameter 18–26 μ.

Type host: *Sorex bendirei bendirei* (Merriam).
Habitat: Small intestine.
Type locality: Humboldt Co., California.
Type slide: U. S. National Museum, Helminthological Collection.

DESCRIPTION

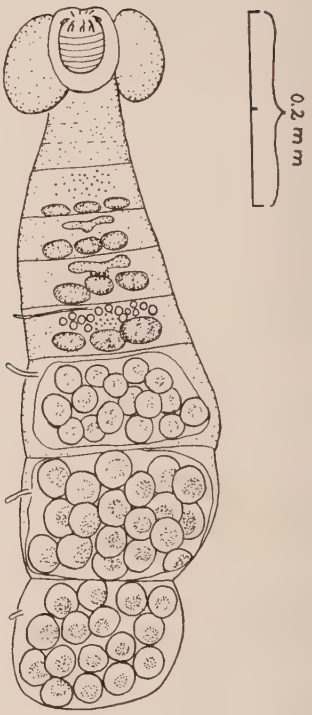
The scolex (Fig. a) bears four suckers with feebly developed musculature; the prominent rostellum is crowned with ten hooks (Fig. d) and has a well developed longitudinal and transverse musculature. Posterior to the scolex there is a very short but distinct neck. The first well defined segment contains testes and a mass of deeply staining granules anterior to the testes. In the second and third segments, the vitelline gland and the ovary are well defined; the genital ducts may be clearly distinguished in the third segment. The fourth segment is usually semigravid with the testes frequently persisting. In some specimens, there is an additional semi-gravid segment. The next two or three segments are gravid, containing nothing but the uterus filled with eggs and the cirrus. The lateral margins of the strobila are smooth (Fig. b). Proglottids are wider than long, except for the terminal gravid segment which may be a little longer than wide. An abrupt increase in size of the segments is seen in the region of gravid proglottids. The genital pores are usually situated in the anterior fourth of the proglottidal margin; in gravid proglottids they may occasionally be seen in the anterior third of the

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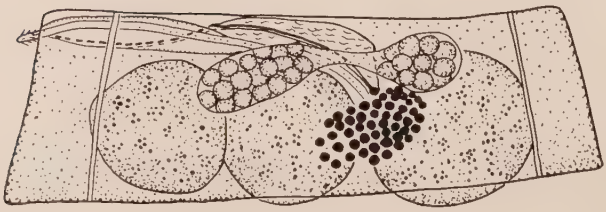


a

0.1 mm



b



c

0.1 mm



d

0.02 mm

Explanation of Plate

All drawing made with the aid of a camera lucida.

Hymenolepis parvissima. Fig. a, scolex; b, whole specimen; c, mature proglottid; d, rostellar hook.

lateral margin. The excretory system apparently consists of two narrow, longitudinal ducts only; these ducts were noted under high magnification and no cross-connections were observed.

Male reproductive system: The testes are arranged in a straight line, posteriorly in the proglottid. Their position did not vary in any of the thirty specimens examined. The testes are frequently ovoid in shape but they may occasionally be spherical (Fig. c). The slender cirrus is covered with small spines. No seminal vesicle was observed.

Female reproductive system: The ovary is a smooth, bilobed structure situated in the anterior half of the segment. Posterior to it is the coarsely granular vitelline gland which may be roughly spherical in some proglottids and irregular in shape in others. Ovary and vitelline gland may be observed in two or three proglottids of the strobila. The vagina can be traced to the region between ovary and vitelline gland; it has a seminal receptacle proximally and narrows to a slender duct about half-way between its origin and the genital pore.

The uterus is sac-like and completely fills the terminal gravid segment. The eggs are spherical to slightly ovoid in outline and have three envelopes.

DISCUSSION

The smallest cestode previously known is *Protogynella blarinae* Jones, 1943 from the shrew *Blarina brevicauda*. According to Jones, this cestode is 750 μ long and 140 μ wide. Locker and Rausch (1952) described another very small cestode, *Hymenolepis sphenomorphus* from *Sorex vagrans,* with a strobilar length up to 800 μ and with about 15 segments per strobila. *H. sphenomorphus* has ten hooks and thus resembles *H. parvissima* not only in size but also in hook number. The differences between these two species are as follows: *H. sphenomorphus* has a cirrus with a terminal knob which is absent in *H. parvissima*. *H. sphenomorphus* also has a smaller scolex, smaller eggs and a longer cirrus sac. The other two species from North American shrews which have ten rostellar hooks are *Hymenolepsis blarinae* and *Hymenolepis parva* both described by Rausch and Kuns (1950). These species are larger in size, have many more proglottids, and bear little resemblance to *H. parvissima*. *H. blarinae* is about 90 mm. long and has a triangular arrangement of the testes. It also differs from *H. parvissima* in the shape of the rostellar hooks. *H. parva* differs from *H. parvissima* in the larger size of the scolex and rostellar hooks, smaller egg size, and in the position of the genital pore.

The lower length limits of mature specimens are not reported for either *Protogynella blarinae* Jones or *Hymenolepis sphenomorphus* Locker and Rausch. The smallest mature specimen of *H. parvissima* was less than one half millimeter long. It would be interesting to know which one of these three species can claim the distinction of attaining the smallest size yet recorded for adult tapeworms.

SUMMARY

A new cestode, *Hymenolepis parvissima* n. sp., is described from the shrew *Sorex bendirei bendirei*. Members of this species belong to the smallest cestodes heretofore described.

Relationships with other similar species are discussed.

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ANOPHELES AZTECUS (HOFFMAN, 1935) A NEW DEFINITIVE HOST FOR THE CYCLICAL TRANSMISSION OF *PLASMODIUM BERGHEI* VINCKE AND LIPS, 1948*

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In the course of a project on experimental malarial chemotherapy in our Laboratory, we were faced with the necessity of obtaining sporozoite-induced *Plasmodium berghei* infections. Attempts were made to infect anopheline mosquitos indigenous to Mexico.

Mosquito infections with *P. berghei* had been described by Ramakrishnan and Prakash (1950) Rodhain and Vincke (1951) Yoeli and Wall (1951, 1952a, 1952b). So far only *Anopheles stephensi, A. maculipennis atroparvus, A. quadrimaculatus,* and *A. gambiae,* had been successfully infected. In this paper we describe the comparative infection of *A. quadrimaculatus, A. aztecus,* and *A. albimanus* with *P. berghei* as well as the cyclical transmission of *P. berghei* with infeced *A. aztecus.*

MATERIALS AND METHODS

The Mukata strain of *P. berghei* was sent to us in June 1952, by the National Institute of Health, U. S. P. H. S., through the courtesy of Doctor Robert Coatney. It has been maintained in our laboratory by weekly blood passages in white rats.

The anophelines used were reared in our colonies at the Institute of Tropical Diseases in Mexico City. *A. aztecus* and *A. albimanus* are from indigenous stock, and *A. quadrimaculatus* was colonized from the N. I. H. strain. *A. aztecus* was maintained at 24° C. and 70 per cent relative humidity and both *A. quadrimaculatus* and *A. albimanus* at 28° C. and 70 per cent R. H.

Hamsters were inoculated with a single dose of 15 million parasites. The parasitemia reached its peak around the eighth day after the inoculation.

The three species of mosquitoes were placed together in one single cage for feeding, and infected hamsters were introduced in the cage every evening at 7 P.M. for two hours, during seven consecutive days. All species fed readily. The engorged females were separated by species, placed in special holding jars and were dissected between the fifth and fourteenth days after feeding.

RESULTS

From the results of our first experiments (table 1) it appears that *A. aztecus* is more susceptible than *A. quadrimaculatus* to the infection by *P. berghei; A. albimanus* being completely insusceptible. The infection occurs in feedings on the fifth to seventh days after inoculation of the hamster, that is before the parasitemia reaches its peak. Our first two attempts to infect anophelines were unsuccessful because feedings were made at and after the eighth day. Three months after the first infections the strain apparently lost its power to infect *A. quadrimaculatus,*

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whereas when the cyclical transmission of *P. berghei* was finally obtained seven months after obtaining the strain, *A. aztecus* was readily infected, with oocyst counts of more than 300 in several stomachs.

We have corroborated the morphological characteristics of the oocyst and sporozite described by other authors. In *A. aztecus* at 24° C. and 70 per cent R. H. the

TABLE 1.—*Infection rates of* P. berghei *in* A. aztecus, A. quadrimaculatus *and* A. albimanus

Hamster, days after inoculation	*A. aztecus*			*A. quadrimaculatus*			*A. albimanus*		
	Dissect.	Inf.	% Inf.	Dissect.	Inf.	% Inf.	Dissect.	Inf.	% Inf.
5	10	7	70	11	3	27	13	0	0
6	10	5	50	1	0	0	12	0	0
7	7	2	28	39	1	2.5	8	0	0
8	22	4	14	49	1	2	20	0	0
9	6	0	0	32	0	0	12	0	0
10	2	0	0	..	..	..	20	0	0
11	2	0	0	..	..	..	5	0	0
12	4	0	0	24	0	0	24	0	0
TOTALS	63	18	28	156	5	3.3	114	0	0

oocysts reached full maturity around the eleventh day, and the sporozoites appeared in the salivary glands at the fourteenth day. The cyclical transmission was obtained by intraperitoneal injection of a macerate of positive *A. aztecus* fourteen days after feeding. Both hamsters and 30-day-old white rats became infected with *P. berghei.*

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MERINGIS RECTUS (SIPHONAPTERA, HYSTRICHOPSYLLIDAE), A NEW ECTOPARASITE OF KANGAROO RATS[1]

HARVEY B. MORLAN

St. Michael's College, Santa Fe, New Mexico

Recognizing the need for further basic biological knowledge to elucidate the epidemiology of rodent-borne diseases such as plague and tularemia, the Communicable Disease Center has established a field ecology study at Santa Fe, New Mexico. The study of the distribution and interrelationships of rodent ectoparasites has been complicated by the discovery of numerous new species. The following description brings the number of known species of the Nearctic genus *Meringis* to thirteen.

Diagnosis and Notes on *Meringis*

The new species is similar to *Meringis arachis* (Jordan, 1929) and belongs to a group of *Dipodomys* parasites which also includes: *Meringis dipodomys* Kohls, 1938; *Meringis bilsingi* Eads and Menzies, 1949; *Meringis nidi* Williams and Hoff, 1951; and *Meringis altipecten* Traub and Hoff, 1951. These six species are alike in lacking the proximal ventral lobe of the ninth sternum in male specimens and in the shape of the body of the spermatheca, which is subcylindrical, in contrast to the pear-shaped spermatheca of other known females of *Meringis*. The movable finger has a broadly rounded apex in *M. dipodomys, M. bilsingi* and *M. nidi* whereas it tapers to a narrow, slightly rounded apex in *M. altipecten, M. arachis,* and the new species. *Meringis altipecten* is described as different from known *Meringis* in the distinctive pronotum which is produced dorsocaudad and bears a strongly curved ctenidium. The male crochet in *M. arachis* (Fig. 9 of Traub and Hoff, 1951) has an acute, slightly upturned apex with a distinctly concave dorsal margin, whereas in the new species the male crochet (Fig. 2) has a broadly rounded apex with nearly parallel dorsal and ventral margins that are straight to slightly convex.

On the basis of the shape of the ninth sternum, *Meringis walkeri* Hubbard, 1940 and *Meringis cummingi* (C. Fox, 1926) might also be placed with this group; however, the female of the former is unknown and the spermatheca of the female ascribed to *M. cummingi* by Hubbard (1940) is described as being inverted pyriform.

A second group of *Meringis* which may be characterized by the presence of a proximal ventral lobe on the ninth sternum of male specimens and a pear-shaped spermatheca include: *Meringis parkeri* Jordan, 1937; *Meringis jamesoni* Hubbard, 1943; *Meringis shannoni* (Jordan, 1929); and *Meringis hubbardi* Kohls, 1938. *Meringis jewetti* Hubbard, 1940, is known only from the male, which is described as having the ninth sternum with a proximal ventral lobe.

Other species of *Meringis* found in Santa Fe County include *M. parkeri, M. jamesoni,* and *M. nidi.* Females of *M. parkeri* and *M. jamesoni* may be easily distinguished under the dissecting microscope by the shape and size of the sper-

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[1] From the Communicable Disease Center, Department of Health, Education, and Welfare, Atlanta, Ga.

matheca. Females of *M. nidi* and the new species have similar appearing spermathecae, but *M. nidi* has a distinct sclerotization anterior to the sinus of sternum VII which is lacking in the new species.

Meringis rectus, new species

Plate I

Head: Dorsally the pre-antennal and post-antennal regions each with the usual three placoid-like structures (dermal pits or pores) plus numerous small punctations. Anterior margin of head evenly rounded, frontoclypeal tubercle absent. Pre-antennal region with about seven small setae irregularly placed between and below a lower row of four large setae and an upper row of three smaller ones. Reduced eye just above base of ctenidium; the broad outer spine overlaps a longer, more slender inner spine. The narrowly rounded apex of genal process extends just beyond the dorsal margin of inner genal spine. Five-segmented labial palpi extend to about three-fourths the length of fore coxae. Maxillary lobe extends to the base of the fourth segment of the maxillary palpi. Scape of antenna with three tiny setae at base and four slightly longer median setae, pedicel with apical setae that do not extend beyond the second segment of the nine-segmented clava. Posterior margin of antennal fossa with ten or twelve small setae. Post-antennal region with an anterior row of three setae, median row of five setae, and posterior row with four small setae placed between five large setae; in each row the seta nearest the antenna is longest and of these three setae the length increases from anterior to posterior.

Thorax: Pronotum with median row of about six large setae and alternating small thin setae on each side. Dorsal margin of pronotum a straight to slightly convex longitudinal line at about the same level as that of the head at the interantennal ridge, pronotum not produced dorsocaudad. Pronotal ctenidium with about seven spines inserted on each side in a nearly transverse line; ventral spine shortest. Anterior portion of mesonotum with scattered small setae, middle portion with two distinct rows of setae, anterior row of medium size, posterior row consisting of larger setae alternating with small thin setae, about halfway between the insertion of caudal row and the posterior margin of the mesonotum are three or four pseudosetae on each side. Mesepisternum with one or two setae. Mesepimere with four or five setae. Metanotum with an anterior row of medium setae and posterior row of larger setae alternating with small thin setae. Lateral metanotal area with one long seta in center of area and another of equal size inserted in area of the heavy dorsal ridge, a third more ventral and smaller seta in one paratype male specimen; ventral ridge relatively undeveloped. Metepisternum with one long and sometimes one tiny seta near heavily sclerotized pleural arch. Metepimere with an anterior row of three of four setae, a second row of four or five, third row represented by a single seta.

Legs: Procoxa with many scattered lateral setae that are longer than the marginal and thinner than the apical setae of the mesocoxa and metacoxa. Inner surface of metacoxa with five to seven spiniforms located just distal to middle of anterior margin. Profemur with about nine small lateral setae, each femora with two long curved setae near apex of posterior margin. Tibiae with long heavy setae along posterior and apical margins. An apical seta of metatibia extends beyond apex of first tarsal segment, of mesotibia beyond second segment, and of protibia to third segment. Metatarsus with distal seta of first segment extending beyond apex of second segment, seta of second segment extending to apex of fourth segment, and seta of third segment extending nearly to apex of fifth segment, setae of mid- and fore-tarsi relatively shorter. Measurements (in microns) of holotype:

	Tibia	Tarsal segments (exclusive of petiolate base)				
		I	II	III	IV	V
Proleg	126	42	42	38	38	80
Mesoleg	235	129	84	49	35	84
Metaleg	315	220	147	80	42	94

Abdomen: Terga with anterior row of medium setae and posterior row of large setae interspersed with small fine setae; without apical spinelets. Basal sternum with one or two setae on ventral margin, apparently without striarium. Unmodified sterna of male usually with four setae on each side, of female usually with an anterior row of one or two setae and a posterior row of four to six setae. With three antepygidial setae; middle seta at least twice (in male about three times) as long as dorsal seta, ventral seta longer than dorsal seta.

Modified Abdominal Segments, Male (Figs. 1, 2, 3): Eighth tergum small, extending ventrally to below apex of proximal arm of ninth sternum, with about seven setae inserted anterior to the spiracle. Eighth sternum large, with four to six large setae on posterior ventral quarter.

Clasper (Fig. 1) large, much broader than movable finger, posterior margin evenly rounded on ventral portion, with a slight concavity below apex, with a small thin acetabular seta above ventral margin of movable finger, with two very long subapical setae and fourteen to eighteen small and medium setae on or near dorsoapical margins. Manubrium long, narrow, slightly sinuate. Movable finger nearly three times as long as broad, tapering to narrowly rounded apex, cephalic margin nearly straight with a small subapical seta, caudal margin convex with a group of about twelve medium setae along central third and three smaller setae near apex, with ten or twelve tiny setae scattered near cephalic and apical margins.

Ninth sternum with proximal arm about one-half as broad as distal arm; proximal arm tapered to blunt apex. Distal arm with two small setae on rounded apex; ventral margin with two short subapical spiniforms, the upper lighter and more slender than the lower, five or six small submarginal setae.

Median dorsal lobes and lateral lobes of the aedeagus elongate, of about equal length; median dorsal lobe bifid, subacuminate, curving ventrad apically; lateral lobes apically blunt and slightly turned downward (Fig. 2). Crochets large, conspicuously broader and longer than the other apical lobes of the aedeagus, apex broadly rounded, nearly parallel dorsal and ventral margins are straight to slightly convex. Penis rods not coiled.

Female (Figs. 4, 5, 6, 7): Seventh sternum with shallow sinus near ventral margin; with posterior row of five setae and anterior row of about seven shorter setae. Eighth tergum with setae beginning below antepygidial setae and extending to the ventral margin, especially numerous along the caudal margin. Eighth sternum oblong, with three to five tiny apical setae. Anal stylet (Fig. 5) more than twice as long as broad, with a long apical seta and two minute subapical setae. Ventral anal lobe (Fig. 7) acuminate, with convex ventral margin. Spermatheca (Fig. 6) with curved tail of about the same length as body; body subcylindrical, ends subequal, slightly thinner at middle. Ductus receptaculi seminis and bursa copulatrix noticeably sclerotized, with a heavily sclerotized, lanceolate ventral structure.

Holotype: Male (ex *Dipodomys spectabilis baileyi* Goldman, Airport, Santa Fe County, New Mexico, March 12, 1952, H. B. Morlan, collector) deposited in the U.S. National Museum, No. 59914.

Allotype: Female (same collection data as holotype) deposited in the U.S. National Museum.

Paratypes: (same collection data as holotype) one male and one female each to Robert Traub, Richard B. Eads, and U. S. Public Health Service laboratories as follows: Rocky Mountain Laboratory, Hamilton, Montana; Communicable Disease Center Museum, Atlanta, Georgia; C.D.C., San Francisco (California) Field Station; Santa Fe (New Mexico) Field Station.

Records: The new flea is a common parasite of *Dipodomys spectabilis baileyi* at all seasons, but is more prevalent during summer months. In contrast, *M. nidi* is absent from the same host during summer and becomes more abundant during the winter months. Detailed data to show seasonal abundance of these two fleas on *D. spectabilis* are given in Table 1.

TABLE 1.—*Infestation of* Dipodomys spectabilis *with* Meringis rectus *and* M. nidi *by months. Airport, Santa Fe County, New Mexico*

1952	Number of examinations	Percent infested with:		Average number of fleas	
		M. rectus	*M. nidi*	*M. rectus*	*M. nidi*
Jan	68	29	91	0.7	6.8
Feb	59	40	90	0.9	6.4
Mar	77	81	79	4.1	6.0
Apr	48	96	83	10.8	3.7
May	46	96	24	10.9	0.8
Jun	40	98	0	7.8	0
Jul	35	89	0	5.1	0
Aug	60	69	0	2.3	0
Sep	55	93	0	7.1	0
Oct	47	98	38	13.9	1.5
Nov	26	81	88	5.5	3.2
Dec	25	16	76	0.2	5.2

At the Airport Study Area of the Santa Fe Field Station, other animals found infested with the new flea include: *Dipodomys ordi medius, Neotoma albigula*

albigula, Neotoma micropus canescens, Onychomys leucogaster melanophrys, Perognathus flavus flavus, Peromyscus maniculatus rufinus, Peromyscus truei truei, Reithrodontomys megalotis aztecus, Citellus spilosoma marginatus, and *Sylvilagus auduboni warreni.*

Specimens of the new species from the collection of the San Francisco Field Station include a single male ex *Sigmodon hispidus* ssp. from Reeves County, Texas; other specimens, all ex *D. spectabilis* ssp., were collected in Texas from Reeves and Winkler Counties, and in New Mexico from Bernalillo, Sandoval, San Juan, Lincoln, Sierra, and Torrence Counties.

Three males of the new species, received from Richard B. Eads, were collected from *D. spectabilis* in Dawson County, Texas. The crochet in these specimens has a mildly sinuate dorsal margin with variation from blunt to subacute apex, but lacks the distinctly concave dorsal margin found in topotype specimens of *M. arachis.*

ACKNOWLEDGEMENTS

Prior to the location of a Field Station at Santa Fe, the presently described flea was recognized as a new species by Frank M. Prince, Officer in Charge, C.D.C. San Francisco Field Station. The author is grateful to him for permission to describe the new flea and for the loan of specimens collected during previous plague surveys.

The contributions, especially in direction of the trapping and determination of mammals, of Robert Holdenried, Officer in Charge, Santa Fe Field Station, are gratefully acknowledged.

Thanks are due to Harold E. Stark, Robert Traub, Richard B. Eads, and Harry D. Pratt for a critical review of the manuscript.

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Explanation of Plate I

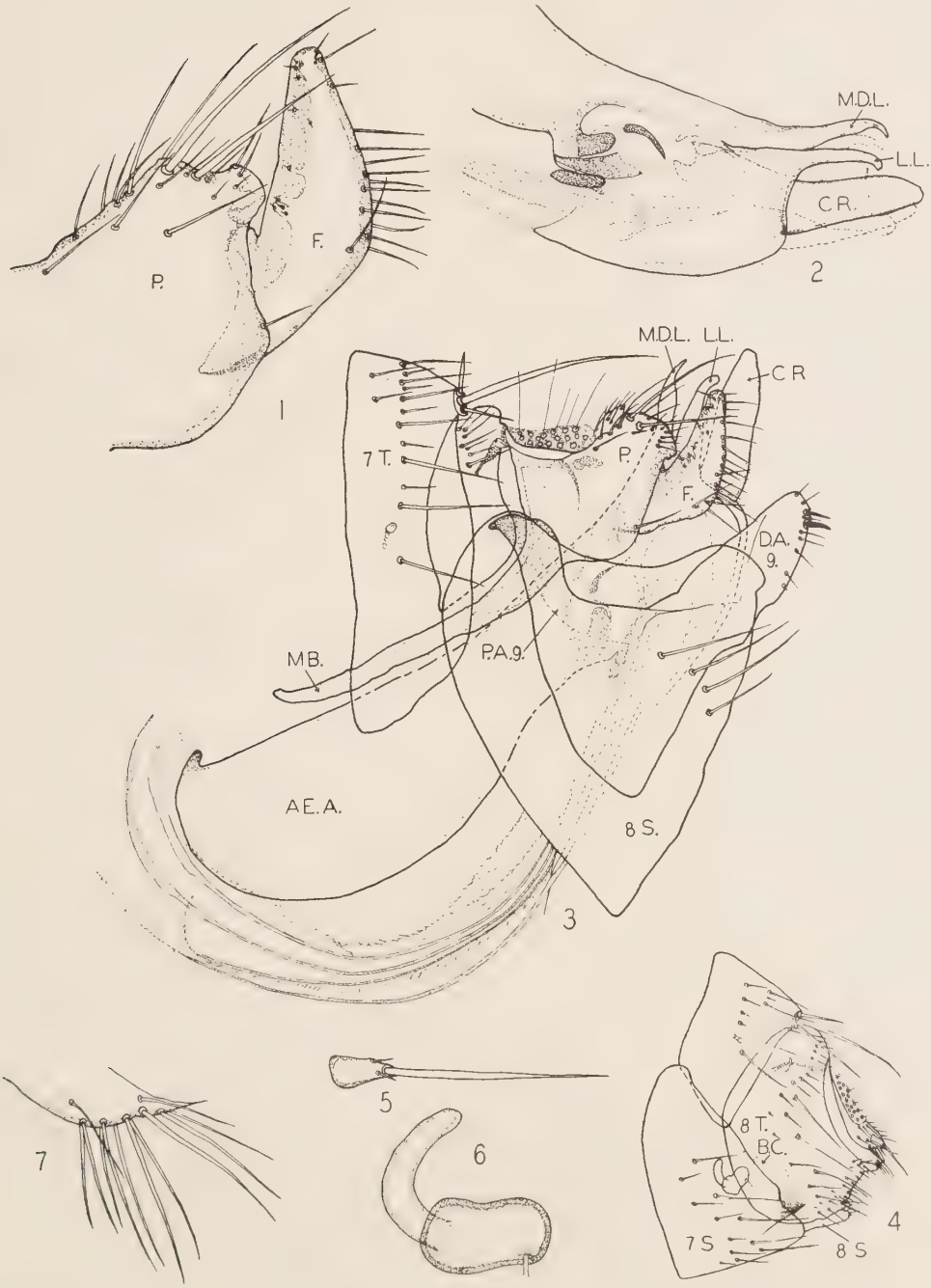
Meringis rectus, new species

Fig. 1. Clasper with movable finger.
Fig. 2. Distal portion of aedeagus.
Fig. 3. Modified abdominal segments, male.
Fig. 4. Modified abdominal segments, female.
Fig. 5. Anal stylet, female.
Fig. 6. Spermatheca.
Fig. 7. Ventral anal lobe, female.

Abbreviations

AE.A., aedeagal apodeme; B.C., bursa copulatrix; CR., crochet of aedeagus; D.A.9, distal arm of ninth sternum; F., movable finger; L.L., lateral lobes of aedeagus; MB., manubrium; M.D.L., median dorsal lobe; P., immovable process of clasper; P.A.9, proximal arm of ninth sternum; 7 S., seventh sternum; 8 S., eighth sternum; 8 T., eighth tergum.

PLATE I



A SYNOPSIS OF NORTH AMERICAN FLEAS, NORTH OF MEXICO, AND NOTICE OF A SUPPLEMENTARY INDEX[1]

WILLIAM L. JELLISON, BETTY LOCKER, AND ROMA BACON

An "Index to the Literature of the Siphonaptera of North America" was published in 1942 by Jellison and Good. References to Mexican species were not included, with a few exceptions, but the known records from Greenland were listed. The 1942 Index included references up to July 1, 1939, and has proved useful to students of flea taxonomy. As much literature on North American fleas has accumulated in the past fifteen years as had been published previously, so the need for a supplement to the 1942 Index is obvious. Supplement I has been completed for the period July 1939 to December 31, 1950. However, because of the rather limited number of workers specializing in this group of parasites, and in view of the cost of formal publication, it has been reproduced by mimeograph, with only fifty copies for distribution. Copies have already been sent to active workers in flea taxonomy in North America and elsewhere, as well as to libraries at the University of Minnesota, University of California, U. S. Department of Agriculture, U. S. National Museum, Camp Detrick (Frederick, Maryland), National Institutes of Health (Bethesda, Maryland), Army Medical Center (Washington, D. C.), Hooper Foundation for Medical Research (San Francisco, California), Communicable Disease Center, P. H. S. (Atlanta, Georgia), Arctic Health Research Center, P. H. S. (Anchorage, Alaska), and the Canadian National Museum (Ottawa, Canada).

The style of Supplement I follows closely that of the 1942 Index, except that notes on host and locality data under each species and the entire section on "Guide to Type Localities" have been omitted. References to descriptions of new species are accompanied by complete data as to sex or sexes described, hosts, and localities. There is still available at the Rocky Mountain Laboratory a generous supply of the 1942 Index. The 1942 Index will be a necessary adjunct to the use of the mimeographed Supplement I.

Several important papers, which contain numerous taxonomic changes on North American fleas, have appeared since the closing of this Supplement I, but the synopsis that follows is based entirely on Supplement I, therefore it is complete only through 1950.

Seventy-two genera containing 243 species and fifty-five subspecies are recognized in the North American faunae north of Mexico, including Greenland. The following synopsis of "families, subfamilies, genera, species, and subspecies" has been prepared to conform with the arrangement used by Holland (1949, "The Siphonaptera of Canada") who acknowledged the help of Dr. Karl Jordan. It will serve to validate a few nomenclatorial changes which appeared in the mimeographed Supplement I and to indicate numerous synonyms, most of which have been previously recorded in literature.

Received for publication, June 22, 1953.

[1] From the U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute, Rocky Mountain Laboratory, Hamilton, Montana.

ORDER SIPHONAPTERA

1. TUNGIDAE

A. TUNGINAE

Tunga Jarocki 1938.
T. penetrans (Linnaeus) 1758.

B. HECTOSYLLINAE

Hectopsylla Frauenfeld 1860.
H. psittaci Frauenfeld 1860.
Rhynchopsyllus Haller 1880.
= *Maxilliopsylla* Schreiter and Shannon 1927, syn.
R. pulex Haller 1880.
= *Maxilliopsylla lilloi* Schreiter and Shannon, 1927, syn.

2. PULICIDAE

A. SPILOPSYLLINAE

Cediopsylla Jordan 1925.
C. inaequalis inaequalis (Baker) 1895.
C. inaequalis interrupta Jordan 1925.
C. simplex (Baker) 1895.
Hoplopsyllus Baker 1905.
H. affinis (Baker) 1904.
H. anomalus (Baker) 1904.
H. foxi Ewing 1924.
= *H. powersi* Fox 1926, syn.
= *H. minutus* Fox 1925, syn.
= *H. tenuidigitus* Steward 1940, new synonymy.
H. glacialis glacialis (Taschenberg) 1880.
H. glacialis lynx (Baker) 1904.

B. PULICINAE

Echidnophaga Olliff 1886.
E. gallinacea (Westwood) 1875.
= *Pulex pullulorum* Johnson 1890.
Juxtapulex Wagner 1933.
J. porcinus (Jordan and Rothschild) 1923.
Pulex Linnaeus 1758.
P. irritans irritans Linnaeus 1758.
= *P. irritans dugesii* Baker 1904, syn.
= *P. simulans* Baker 1895, syn.

C. ARCHAEOPSYLLINAE

Ctenocephalides Stiles and Collins 1930.
C. canis (Curtis) 1826.
C. felis felis (Bouché) 1835.

D. XENOPSYLLINAE

Xenopsylla Glinkiewicz 1907.
X. cheopis (Rothschild) 1903.
= *X. pachyuromyidis* Glinkiewicz 1907, syn.

3. MALACOPSYLLIDAE

A. RHOPALOPSYLLINAE

Polygenis Jordan 1939.
P. gwyni (Fox) 1914.
= *Rhopalopsyllus sigmodoni* Stewart 1930, syn.
Rhopalopsyllus Baker 1905.
R. cacicus Jordan and Rothschild 1908.
= *R. coxi* Eads 1946, syn.

4. VERMIPSYLLIDAE

Arctopsylla Wagner 1930.
A. setosa (Rothschild) 1906.

A. ursi (Rothschild) 1902.
Chaetopsylla Kohaut 1903.
C. floridensis (Fox) 1939.
C. globiceps (Taschenberg) 1880.
C. lotoris (Stewart) 1926.

5. HYSTRICHOPSYLLIDAE

A. HYSTRICHOPSYLLINAE

Atyphloceras Jordan and Rothschild 1915.
A. artius Jordan 1933.
A. echis Jordan and Rothschild 1915.
A. felix Jordan 1933.
A. longipalpus Stewart 1940.
A. multidentatus (Fox) 1909.
Hystrichopsylla Taschenberg 1880.
H. dippiei Rothschild 1902.
H. gigas (Kirby) 1837.
H. mammoth Chapin 1921.
H. occidentalis Holland 1949.
H. schefferi Chapin 1919.
H. spinata Holland 1949.
H. tahavuana Jordan 1929.
Saphiopsylla Jordan 1931.
S. bishopi (Jordan) 1933.
Stenoponia Jordan and Rothschild 1911.
S. americana (Baker) 1899.
= *S. wetmorei* Chapin 1919, syn.

B. NEOPSYLLINAE

Catallagia Rothschild 1915.
C. borealis Ewing 1929.
C. chamberlini Hubbard 1940.
C. charlottensis (Baker) 1898.
C. decipiens Rothschild 1915.
C. mathesoni Jameson 1950.
C. moneris Jordan 1937.
C. motei Hubbard 1940.
C. onaga Jordan 1929.
C. rutherfordi Augustson 1941.
C. sculleni Hubbard 1940.
C. vonbloekeri Augustson 1941.
C. wymani (Fox) 1909.
Delotelis Jordan 1937.
D. telegoni (Rothschild) 1905.
Epitedia Jordan 1938.
E. faceta (Rothschild) 1915.
E. neotomae Jameson 1946.
E. scapani Wagner 1936.
= *E. jordani* Hubbard 1940, syn.
= *E. stewarti* Hubbard 1940, syn.
E. stanfordi Traub 1944.
E. testor (Rothschild) 1915.
E. wenmanni (Rothschild) 1904.
= *Neopsylla similis* Chapin 1919.
Meringis Jordan 1937.
= *Atheropsylla* Stewart 1940, syn.
M. arachis Jordan 1929.
M. bilsingi Eads and Menzies 1949.
M. cummingi Fox 1926.
= *Atheropsylla bakeri* Stewart 1940, syn.
M. dipodomys Kohls 1938.
M. hubbardi Kohls 1938.
M. jamesoni Hubbard 1943.

M. jewetti Hubbard 1940.
M. parkeri Jordan 1937.
M. shannoni (Jordan) 1929.
M. walkeri Hubbard 1940.
Neopsylla Wagner 1903.
N. inopina Rothschild 1915.
N. texana Stewart 1930.
Phalacropsylla Rothschild 1915.
P. allos Wagner 1936.
P. monticola Augustson 1941.
P. paradisea Rothschild 1915.
Tamiophila Jordan 1938.
T. grandis (Rothschild) 1902.

C. RHADINOPSYLLINAE

Actenophthalmus Fox 1925.
A. heiseri (McCoy) 1911.
Micropsylla Dunn and Parker 1923.
M. sectilis goodi Hubbard 1941.
M. sectilis sectilis (Jordan and Rothschild) 1923 (Nov. 20).
= *M. peromyscus* Dunn 1923 (Nov. 23), syn.
Paratyphloceras Ewing 1940.
P. oregonensis Ewing 1940.
Rectofrontia Wagner and Argyropulo 1934.
R. fraterna (Baker) 1895.
= *Neopsylla hamiltoni* Dunn 1923, syn.
R. spinulosa Good 1941?
of Hubbard 1943, *nomen nudem*
Trichopsylloides Ewing 1938.
= *Phaneris* Jordan 1939, syn.
T. oregonensis Ewing 1938.
= *Phaneris hubbardi* Jordan 1939, syn.

D. CTENOPHTHALMINAE

Carteretta Fox 1927.
= *Trirachipsylla* Stewart 1940, syn.
C. carteri carteri Fox 1927.
= *Trirachipsylla digitiformis* Stewart 1940, syn.
C. carteri clavata Good 1942.
Corrodopsylla Wagner 1929.
C. curvata curvata (Rothschild) 1915.
C. curvata obtusata (Wagner) 1929.
= *Doratopsylla jellisoni* Hubbard 1940, syn.
C. hamiltoni (Traub) 1944.
Ctenophthalmus Kolenati 1856.
C. pseudagyrtes pseudagyrtes Baker 1904.
Doratopsylla Jordan and Rothschild 1912.
D. blarinae Fox 1914.

E. ANOMIOPSYLLINAE

Anomiopsyllus Baker 1904.
A. amphibolus Wagner 1936.
A. congruens Stewart 1940.
A. falsicalifornicus Fox 1926.
A. hiemalis Eads and Menzies 1948.
A. montanus Collins 1936.
A. nudatus (Baker) 1898.
= *A. californicus* Baker 1904, syn.
Callistopsyllus Jordan and Rothschild 1915.
C. campestris Holland 1949.
C. deuterus Jordan 1937.
C. terinus (Rothschild) 1905.
= *C. paraterinus* Wagner 1940, syn.
Conorhinopsylla Stewart 1930.

C. nidicola Jellison 1945.
C. stanfordi Stewart 1930.
Megarthroglossus Jordan and Rothschild 1915.
M. bisetis Jordan and Rothschild 1915.
M. divisus divisus (Baker) 1898.
= *Pulex longispinus* Baker 1895, preoccupied.
M. divisus exsecatus Wagner 1936.
M. divisus wallowensis Hubbard 1947.
M. procus oregonensis Hubbard 1947.
M. procus procus Jordan and Rothschild 1915.
M. pygmaeus Wagner 1936.
M. sicamus Jordan and Rothschild 1915.
M. similis Wagner 1936.
M. spenceri Wagner 1936.
Stenistomera Rothschild 1915.
S. alpina (Baker) 1895.
= *Delotelis mohavensis* Augustson 1941, syn.
S. macrodactyla Good 1942.

F. NEARCTOPSYLLINAE

Corypsylla Fox 1908.
C. jordani Hubbard 1940.
C. ornata Fox 1908.
C. setosifrons Stewart 1940.
Corypsylloides Hubbard 1940.
C. kohlsi (Hubbard) 1940 (April).
= *C. spinata* Fox 1940 (June), syn.
Nearctopsylla Rothschild 1915.
N. brooksi (Rothschild) 1904.
N. genalis genalis (Baker) 1904.
= *Ctenopsylla hygini* Rothschild 1904 in part, syn.
N. genalis hygini (Rothschild) 1904.
N. genalis laurentina Jordan and Rothschild 1923.
N. hamata Holland and Jameson 1950.
N. hyrtaci (Rothschild) 1904.
N. jordani jordani Hubbard 1940 (April).
= *N. hygini columbiana* Wagner 1940 (May), syn.
N. jordani traubi Hubbard 1949.
N. princei Holland and Jameson 1950.

6. CERATOPHYLLIDAE

A. AMPHIPSYLLINAE

Amphipsylla Wagner 1908.
A. ewingi Fox 1940.
A. sibirica pollionis (Rothschild) 1905.
Ctenophyllus Wagner 1927.
C. terribilis (Rothschild) 1903.
Geusibia Jordan 1938.
= *Augustsonius* Hubbard 1941, syn.
G. ashcrafti Augustson 1940.
Odontopsyllus Baker 1905.
O. dentatus (Baker) 1904.
= *Ceratophyllus ponerus* Rothschild 1909, syn.
= *O. spenceri* Dunn 1923, syn.
O. multispinosus (Baker) 1898.

B. DOLICHOPSYLLINAE

Dolichopsyllus Baker 1905.
D. stylosus (Baker) 1904.

C. CERATOPHYLLINAE

Amphalius Jordan 1933.
A. necopinus (Jordan) 1925.

Ceratophyllus Curtis 1832.
C. adustus Jordan 1932.
C. borealis Rothschild 1907.
C. celsus celsus Jordan 1926.
C. diffinis Jordan 1925.
= *C. rileyi* Liu 1935, syn.
C. gallinae (Schrank) 1803.
= *C. gibsoni* Fox 1914, syn.
C. garei Rothschild 1902.
= *C. quebecensis* Fox 1940, syn.
= *C. utahensis* Chapin 1919, syn.
C. idius Jordan and Rothschild 1920.
C. niger inflexus Jordan 1929.
C. niger niger Fox 1908.
C. petrochelidoni Wagner 1936.
C. riparius Jordan and Rothschild 1920.
C. swansoni Liu 1935.
C. tundrensis Holland 1944.
C. vagabundus vagabundus (Boheman) 1866.
Dactylopsylla Jordan 1929.
D. bluei (Fox) 1909.
= *Ceratophyllus stimsoni* Fox 1914, syn.
D. bottaceps Hubbard 1943.
D. comis Jordan 1929.
D. monticola Prince 1945.
D. moorei Hubbard 1949.
D. neomexicana Prince 1945.
D. nuditenacula Prince 1945.
D. pacifica Hubbard 1943.
D. percernis Eads and Menzies 1949.
D. rara Fox 1940.
Dasypsyllus Baker 1905.
D. gallinulae perpinnatus (Baker) 1904.
D. stejnegeri (Jordan) 1929.
Diamanus Jordan 1933.
D. montanus (Baker) 1895.
= *Ceratophyllus acutus* Baker 1904, syn.
Foxella Wagner 1929.
F. ignota acuta Stewart 1940.
F. ignota albertensis (Jordan and Rothschild) 1915.
F. ignota apachina (Fox) 1914.
F. ignota arizonensis Hubbard 1947.
F. ignota clantoni Hubbard 1949.
F. ignota coufferi Augustson 1942.
F. ignota franciscana (Rothschild) 1910.
F. ignota ignota (Baker) 1895.
= *Typhlopsylla americana* Baker 1895 in part, syn.
F. ignota omissa Prince 1945.
F. ignota recula (Jordan and Rothschild) 1915.
F. ignota utahensis Wagner 1936.
Jellisonia Traub 1944 (Dec.).
= *Pleochaetoides* Augustson 1945 (Jan.).
J. bullisi (Augustson) 1945.
J. ironsi (Eads) 1946.
Malaraeus Jordan 1933.
M. bitterrootensis (Dunn) 1923.
= *Ceratophyllus isus* Jordan 1925, syn.
M. dobbsi Hubbard 1940.
M. eremicus (Baker) 1904.
M. euphorbi (Rothschild) 1905.
M. neotomae (Fox) 1940, new combination.
M. penicilliger dissimilis Jordan 1938.
M. sinomus (Jordan) 1925.

M. telchinum (Rothschild) 1905.
Megabothris Jordan 1933.
M. abantis (Rothschild) 1905.
= *M. adversus* Wagner 1936, syn.
M. acerbus (Jordan) 1925.
M. asio asio (Baker) 1904.
M. asio megacolpus (Jordan) 1929.
= *M. asio orectus* Jordan 1938, syn.
M. atrox Jordan 1925.
M. calcarifer gregsoni Holland 1950.
M. clantoni clantoni Hubbard 1949 (June).
= *M. obscurus* Holland 1949 (Sept.).
M. clantoni johnsoni Hubbard 1949.
M. groenlandicus (Wahlgren) 1903.
M. immitis (Jordan) 1929.
M. lucifer (Rothschild) 1905.
M. princei Hubbard 1949.
M. quirini (Rothschild) 1905.
Mioctenopsylla Rothschild 1922.
= *Boreopsyllus* Ewing 1929, syn.
M. arctica Rothschild 1922.
= *Boreopsyllus hadweni* (Ewing) 1927, syn
Monopsyllus Kolenati 1857.
M. anisus (Rothschild) 1907.
M. ciliatus ciliatus (Baker) 1904.
M. ciliatus kincaidi Hubbard 1947.
M. ciliatus mononis (Jordan) 1929.
M. ciliatus protinus (Jordan) 1929.
M. eumolpi americanus Hubbard 1950.
M. eumolpi canadensis Hubbard 1949.
M. eumolpi charlestonensis Hubbard 1950.
M. eumolpi cyrturus (Jordan) 1929.
M. eumolpi eumolpi (Rothschild) 1905.
M. eumolpi wallowensis Hubbard 1950.
M. eutamiadis Augustson 1941.
M. exilis exilis (Jordan) 1937.
M. exilis kansensis Hubbard 1943.
M. exilis opadus Jordan 1938.
M. exilis triptus Jordan 1938.
M. fornacis Jordan 1937.
M. thambus (Jordan) 1929.
= *Ceratophyllus bakeri* Wagner 1935, syn.
M. vison (Baker) 1904.
= *Ceratophyllus lucidus* (Baker) 1904, syn.
M. wagneri kylei Hubbard 1949.
M. wagneri ophidius (Jordan) 1929.
M. wagneri systaltus (Jordan) 1929.
M. wagneri wagneri (Baker) 1904.
= *Ceratophyllus peromysci* Stewart 1928, syn.
Nosopsyllus Jordan 1933.
N. fasciatus (Bosc) 1801.
= *Ceratophyllus californicus* Baker 1904, syn.
= *Ceratophyllus canadensis* Baker 1904, syn.
= *Ceratophyllus oculatus* Baker 1904, syn.
N. londiniensis (Rothschild) 1903.
Opisocrostis Jordan 1933.
O. bruneri (Baker) 1895.
= *Ceratophyllus saundersi* Jordan 1933, new synonymy.
O. hirsutus (Baker) 1895.
O. labis (Jordan and Rothschild) 1922.
O. oregonensis Good and Prince 1939.
O. tuberculatus cynomuris Jellison 1939.
= *O. ornatus* Fox 1940, syn.

O. tuberculatus tuberculatus (Baker) 1904.
O. washingtonensis Good and Prince 1939.
Opisodasys Jordan 1933.
O. enoplus (Rothschild) 1909.
O. keeni keeni (Baker) 1896.
O. keeni nesiotus Augustson 1941, new combination.
O. pseudarctomys (Baker) 1904.
= *Ceratophyllus acasti* (Rothschild) 1905, syn.
O. robustus robustus (Jordan) 1925.
= *O. spatiosus* Fox 1940.
O. vesperalis (Jordan) 1929.
= *O. jellisoni* Fox 1941, in part, syn.
Orchopeas Jordan 1933.
= *Bakerella* Wagner 1930, preoccupied.
O. caedens caedens (Jordan) 1925.
O. caedens durus (Jordan) 1929.
O. dieteri (Fox) 1929.
O. howardii howardii (Baker) 1895.
= *O. wickhami* (Baker) 1895, syn.
= *Pulex gillettei* Baker 1895, syn.
O. howardii texensis Eads 1950.
O. labiatus (Baker) 1904.
O. latens (Jordan) 1925.
O. leucopus (Baker) 1904.
= *Ceratophyllus aeger* Rothschild 1905, syn.
O. nepos (Rothschild) 1905.
O. sexdentatus agilis (Rothschild) 1905.
O. sexdentatus cascadensis Jordan 1939.
O. sexdentatus intermedius Hubbard 1943.
O. sexdentatus neotomae Augustson 1943.
O. sexdentatus nevadensis (Jordan) 1929.
O. sexdentatus pennsylvanicus (Jordan) 1928.
O. sexdentatus schisintus (Jordan) 1929.
O. sexdentatus sexdentatus (Baker) 1904.
Oropsylla Wagner and Ioff 1926.
= *Aetheopsylla* Stewart and Holland 1940, syn.
O. alaskensis (Baker) 1904.
O. arctomys (Baker) 1904.
= *Aetheopsylla septentrionalis* Stewart and Holland 1940, syn.
O. idahoensis (Baker) 1904.
= *Ceratophyllus pocantis* Rothschild 1905, syn.
= *Ceratophyllus bertholfi* Fox 1927, syn.
O. rupestris (Jordan) 1929.
Pleochaetis Jordan 1933.
P. sibynus (Jordan) 1925.
Tarsopsylla Wagner 1927.
T. coloradensis (Baker) 1895.
= *Opisodasys jellisoni* Fox 1941, syn. in part.
Thrassis Jordan 1933.
= *Thrassoides* Hubbard 1947, syn.
Th. acamantis (Rothschild) 1905.
Th. aridis Prince 1944.
Th. arizonensis arizonensis (Baker) 1898.
= *Ceratophyllus proximus* Baker 1904, syn.
Th. arizonensis desertorum Stewart 1937.
Th. arizonensis littoris (Jordan) 1929.
Th. augustsoni Hubbard 1949.
Th. bacchi (Rothschild) 1905.
Th. brennani Augustson 1944.
Th. campestris Prince 1944.
Th. fotus (Jordan) 1925.
= *Ceratophyllus citelli* Stewart 1928, syn.
Th. francisi (Fox) 1927.

Th. gladiolis caducus (Jordan) 1930.
Th. gladiolis gladiolis (Jordan) 1925.
Th. gladiolis johnsoni Hubbard 1949.
Th. hoffmani (Hubbard) 1949.
Th. howelli howelli (Jordan) 1925.
Th. howelli utahensis Wagner 1936.
Th. jellisoni Hubbard 1940.
Th. pandorae Jellison 1937.
Th. pansus (Jordan) 1925.
Th. petiolatus (Baker) 1904.
Th. princei Hubbard 1943.
Th. rockwoodi Hubbard 1942.
Th. setosis Prince 1944.
Th. spenceri Wagner 1936.
Th. stanfordi Wagner 1936.
Leptopsylla Jordan and Rothschild 1911.
= *Ctenopsyllus* Kolenati 1862, preoccupied.
L. segnis (Schönherr) 1811.
= *Pulex musculi* Dugés 1832, syn.
= *Typhlopsylla mexicana* Baker 1896, syn.
Peromyscopsylla Fox 1939.
P. adelpha (Rothschild) 1915.
P. catatina (Jordan) 1928.
P. duma Traub 1944.
P. ebrighti (Fox) 1926.
P. hamifer hamifer (Rothschild) 1906.
P. hamifer longiloba (Jordan) 1939.
P. hamifer markworthi Hubbard 1949.
P. hamifer vigens (Jordan) 1937.
P. hesperomys hemisphaerium Stewart 1940.
P. hesperomys hesperomys (Baker) 1904.
= *P. spinifrons* Fox 1939, syn.
P. hesperomys pacifica Holland 1949.
P. ravalliensis (Dunn) 1923.
P. selenis (Rothschild) 1906.

7. ISCHNOPSYLLIDAE

Eptescopsylla Fox 1940.
E. chapini (Jordan) 1929.
E. vancouverensis (Wagner) 1936.
Myodopsylla Jordan and Rothschild 1911.
M. collinsi Kohls 1937.
= *M. diasi* Costa Lima 1938, syn. (from Mexico).
M. crosbyi (Baker) 1905.
M. gentilis Jordan and Rothschild 1921.
M. insignis (Rothschild) 1903.
= *M. subulata* Chapin 1919, syn.
Myodopsylloides Augustson 1941.
M. palposus (Rothschild) 1904.
= *M. piercei* Augustson 1941, syn.
Sternopsylla Jordan and Rothschild 1921.
= *Aptilopsylla* Ewing 1940, syn.
S. texana (Fox) 1914.
= *Aptilopsylla carlsbadensis* Ewing 1940, new synonymy.

THREE PARASITIC ISOPODS (BOPYRIDAE) FROM THE CAROLINA COAST

A. S. Pearse

Duke University

Mr. Stephen Wainwright presented the writer with males and females of three species of Bopyrids that were collected during trawling expeditions from Beaufort, N. C. One of these species was new and another furnished a specimen of a male, previously unknown.

Bopyrina abbreviata Richardson, 1904

Five females and a male were taken from the branchial cavities of shrimps, *Hippolyte pleurocantha* (Stimpson). Mature females measured 2 mm. in length; the male, 0.7 mm.

Bopyrina urocaridis Richardson, 1904

A male and a female were taken from *Urocaris longicaudata* Stimpson, the same host as Richardson's specimens. The female measures 3 mm. in length and 1.7 mm. in width. As the male has not been known, a brief description is given (Fig. 1).

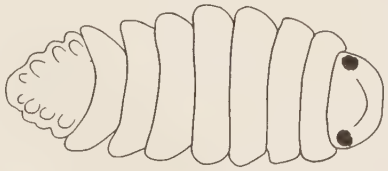


Fig. 1. *Bopyrina urocardis* Richardson. Male.

Male: Length of body 0.7 mm., width 0.2 mmm. Head almost twice as wide as long, truncate in front. Eyes large, 1/7 as wide as head. First thoracic segment shortest; third longest. All thoracic segments with rounded postero-lateral angles; last segment with lateral regions bent posteriad. Abdomen unsegmented but with lateral indentations that indicate four segments. Four pairs of rounded rudimentary appendages on abdomen. No separate telson; posterior margin of abdomen slightly rounded. Head 2/3 as long as abdomen, which is 9/11 as long as wide.

The male of this species differs from that of *B. abbreviata* in having a shorter abdomen of a different shape and with unilateral differences in segmentation. It and the female were collected on Shackelford Banks, July 14, 1952.

Allotype: U. S. Nat. Mus. No. 95118.

Bopyrina crangona n. sp.

A female and accompanying male were taken from the gill cavity of a little shrimp, *Crangon formosus* (Gibbs), collected on Shackleford Banks, July 10, 1952.

Female: Length 5.5 mm., width 2.7 mm.; length of head 0.17 mm., width 0.25 mm. Body asymmetrical, the right side longer than the left. Head turned slightly toward the right; frontal margin nearly straight. Eyes small, distinct.

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Thoracic segments distinct. Epimera slender. Legs present on both lateral margins; rather slender (Fig. 3). Terminal hooks stout and strongly curved. Propodus rather slender. Merus triangular, longer than wide; setose at tip and at base.

Abdominal segments completely fused. Segments more strongly indicated on the left than on the right side (Fig. 4). Four pairs of rudimentary legs also larger on the left. Fifth legs lacking. End of abdomen slightly notched.

Type: U. S. Nat. Mus. No. 95120.

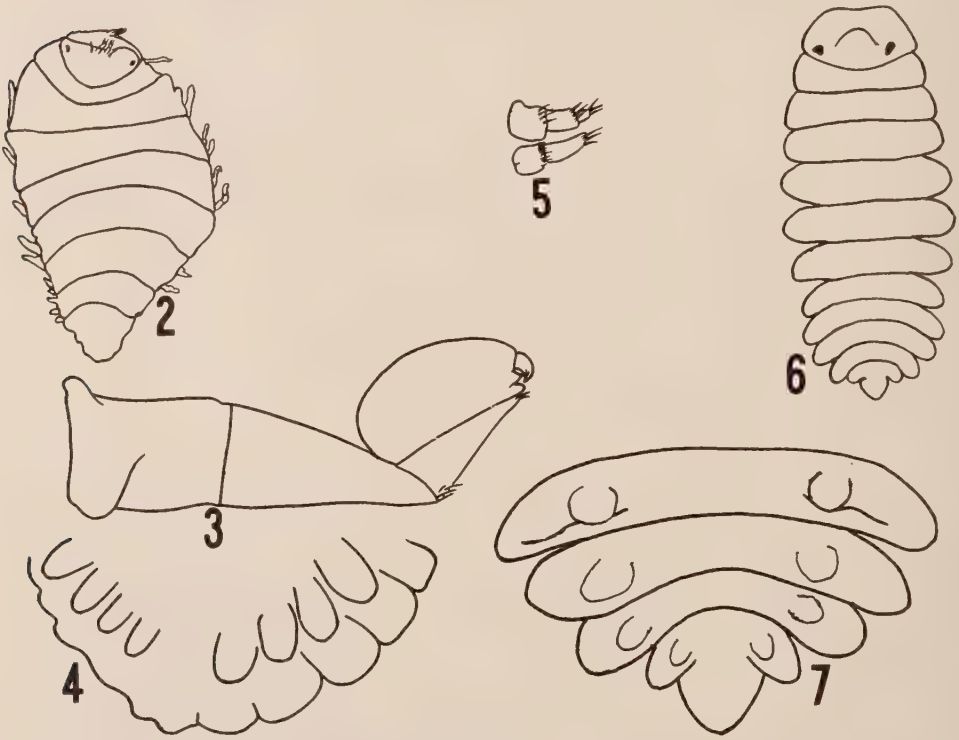
Male: Length 1.1 mm., width 0.5 mm.; length of head 0.1 mm., width 0.2 mm.

Head twice as wide as long; slightly indented in front. First antenna 3-segmented; second antenna 2-segmented (Fig. 5).

Thoracic segments distinct; the first three with blunt lateral margins; the next three with more distinct lateral margins; the last segment with lateral margins curved somewhat posteriorly. All thoracic segments with sub-chelate legs similar to those of the female.

Abdomen with first three segments distinct; the fourth fused with the terminal segment. The abdomen thus consists of four distinct segments. The last apparently consists of a segment with fused pair of appendages and a telson (Fig. 7).

Type: U. S. Nat. Mus. No. 95121.



FIGS. 2–7. *Bopyrina crangona* n. sp. 2–4, Female: 2, dorsal view; 3, fourth leg; 4, ventral view of abdomen 5–7, Male: 5, first and second antennae; 6, dorsal view of whole body, 7, abdomen, ventral view.

This species differs from others in the genus in the following characters: the head of the male is indented on the anterior margin; the abdominal appendages are rounded and unsegmented in both sexes; the female abdomen is unsegmented and that of the male shows three free segments.

REFERENCE

RICHARDSON, HARRIET 1904 Contributions to the natural history of the Isopoda. Part 1. Proc. U. S. Nat. Mus. **27**: 1–89.

A STUDY OF THE SEASONAL CYCLE OF A PROTEOCEPHALAN CESTODE, *PROTEOCEPHALUS STIZOSTETHI* HUNTER AND BANGHAM, FOUND IN THE YELLOW PIKEPERCH, *STIZOSTEDION VITREUM VITREUM* (MITCHILL)*[1]

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Specimens of pikeperch were examined for parasites at monthly intervals from November of 1949 until June of 1951. The adult form of the cestode, *Proteocephalus stizostethi,* was found only in the late spring and early summer and disappeared from this host sometime between early August and mid-September. Small immature specimens of the parasite, which increased in size and maturity during the fall and winter, were then found. Embryonated eggs occured again late in the following spring.

Seasonal cycles of fish tapeworms have been reported by several of the earlier workers. Meggitt (1914) in his study of *Proteocephalus filicollis* found a considerably smaller number of infected fish in the winter, and the adult parasites were rare. The proportion of adults increased again in the spring. Wagner in 1917, as reported by Essex (1927), found only immature forms of *P. torulosus* in the winter, and mature tapeworms between spring and autumn. Essex found in his work on *Corallobothrium giganteum* and *C. fimbriatum* that the adult parasites appeared in the late spring or early summer, reached a maximum during June and July, and disappeared entirely in the latter part of October or early in November. In her work with *Triaenophorus crassus* in *Esox lucius* from Canadian lakes, Ekbaum (1937) found gravid parasites late in May and immature forms from the last of June until the following spring. Miller (1943) in his extensive studies on *Triaenophorus crassus* and *T. nodulosus* from northern pike in Canada, found a similar seasonal cycle. He estimated the state of ripeness of the tapeworms by placing the worms in water; ripe worms released a cloud of eggs from their uterine pores, while unripe worms released no eggs or only a few after several hours. This was referred to as the spawning time of the parasite.

The findings of this study on *P. stizostethi* appear to approximate the results of these other workers very closely.

MATERIALS AND METHODS

All of the fish examined were of legal size and, according to the local fish marketer, came from Lake Erie. The fish had been dead for about three days before examination; yet, in most cases the parasites were found to be vigorously alive. None of the fish had been held in live-tanks previous to their sale. Whole fish were examined at the start of this study, but in most cases only the viscera were available.

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[1] Submitted to the Graduate School in partial fulfillment for the degree of Master of Science. The writer wishes to express his appreciation to Dr. W. D. Lindquist of the Department of Bacteriology and Public Health, Michigan State College for his assistance in this study.

[2] Now at Purdue University, Department of Biological Sciences.

The intestine, stomach, swim bladder, liver, and other internal organs were placed in glass dishes in 0.7 per cent sodium bicarbonate solution, teased apart, and inspected over a good light source. The cestodes were washed in fresh bicarbonate solution and then fixed in hot A.F.A. fixative. After remaining in the fixative for a period of 12 to 24 hours, they were washed in 70 per cent alcohol and stored in fresh alcohol.

Semichons' acetic carmine or a mixture of Delafield's and Ehrlich's hematoxylin was used for whole mount staining. More consistent results were obtained with the carmine stain, but better definition of the internal structures, especially the smaller ducts, was obtained from the hematoxylin stain. Synthetic oil of wintergreen was used as a clearing agent. Because of the rather dense longitudinal musculature of these cestodes, the best method of preparation was to make frontal serial sections of the proglottids. These were made 20 micra in thickness and stained with Harris' hematoxylin and eosin. The clearest and most useful preparations were made by this method.

RESULTS

Material from 75 fish, including whole fish and viscera only, was examined. A total of 189 "in toto" slides were made. Serial sections were prepared from *P. stizostethi* in progressive stages of maturity for a total of 36 slides.

TABLE 1.—*Seasonal variation in the state of the sex organs and in the number of* Proteocephalus stizostethi

Date examined	Number of of fish examined	Number of *P. stizostethi* per fish	Sexual development
1949:			
Nov. 18	2	4	Genital organs developing
Dec. 23	2	14	Mature genitalia; no eggs found
1950:			
Jan. 31	5	9.8	Mature genitalia; no eggs found
Feb. 8	2	7	Mature genitalia; no eggs found
Mar. 23	2	6.5	No eggs in uterus; outlines of uterine pouches seen
Apr. 11	4	9.5	No eggs in uterus; uterine pouches further developed
May 10	5	4.6	Some eggs in uterus; embryos not yet outlined
June 30	3	4	Gravid proglottids; outlines of embryos seen
Aug. 3 through Sept. 13	5	0	No proteocephalids found
Sept. 18	2	2	Immature forms found; no trace of genitalia
Sept. 29	5	9	First rudiments of genitalia seen
Oct. 6	4	1	Genital organs developing
Oct. 27	5	2	Genital organs developing
Nov. 3	4	4	Genitalia further developed
Nov. 17	2	9	Genitalia mature; no eggs found
Dec. 8	2	1.5	Genitalia mature; no eggs found
Dec. 20	3	6	Genitalia mature; no eggs found
1951:			
Jan. 16	2	4	Genitalia mature; no eggs found
Jan. 23	3	6	Genitalia mature; no eggs found
Feb. 8	2	6.5	Genitalia mature; no eggs found
Feb. 23	2	4.5	Genitalia mature; no eggs found
Mar. 9	3	11	Outlines of uterine pouches seen; no eggs found
Mar. 23	3	6	Outlines of uterine pouches seen; no eggs found
Apr. 20	3	3	Embryonated eggs found in uterine pouches
June 30	4	5	Viable embryonated eggs first found

Table 1 shows the observations of the seasonal variation and number of worms found. It seems evident from the data presented that the adult form of this parasite appeared only in the late spring and early summer months and disappeared from the pikeperch some time between the first part of August and mid-September. No specimens of *P. stizostethi* were found in the fish at this time. Small immature

parasites were then found until late September, when worms showing the first rudiments of genitalia were observed. Progressively larger and more mature specimens were seen throughout the fall and winter months, but no eggs or uterine development were observed during this time. The first outlines of uterine pouches, devoid of eggs, were seen in March, and some eggs were seen in these uterine pouches late in April, 1951, and early in May, 1950.

Viable embryonated eggs, which were shed from the ventral openings when the worms were placed in water, were first seen in June of 1951. As late as June 30, 1950, eggs were found in these cestodes, but they were still immature as the onchospheres had not developed by this time.

The above observations also seem to confirm the opinion of La Rue (1914) that in the family PROTEOCEPHALIDAE the outpocketing or lateral branching of the uterus occurs prior to the discharge of eggs into the uterus and is not due to crowding by these eggs.

The size and maturity of the tapeworms secured from fish on any particular date were quite constant except for a period from early October until late in November. During this time tapeworms from the same fish varied in size and maturity from small forms showing only the rudiments of genital organs to larger worms with well developed ovaries and testes. This variation was possibly due to intermittent feeding on the intermediate host at this time. Later in the winter the pikeperch no longer seemed to receive new infections of the parasite. This suggests that the intermediate host was not available in the diet of the pikeperch at that time.

It was not thought that the tapeworms matured and were lost from the fish host at any time excepting the late spring and summer months, as evidence of egg production was not found at any other season. Also, it was not probable that proglottids or sections of the strobila containing eggs were lost from the host during other times of the year since the distinctive end proglottid was found on each specimen of *P. stizostethi* examined. This end proglottid was described by La Rue (1914) as retaining certain of the characteristics of the posterior end of the plerocercoid larva which was not lost in the passage of the young tapeworm through the stomach of the host. It is pointed posteriorly and at the tip has a median excretory pore.

There seemed to be no correlation between the number of *P. stizostethi* found and the season.

SUMMARY

A study of a seasonal cycle for a period of two years of the fish tapeworm, *Proteocephalus stizostethi* Hunter and Bangham, 1933, found in the yellow pikeperch (walleye), *Stizostedion vitreum vitreum* (Mitchill), from Lake Erie is presented. Viable embryonated eggs were found only in June. The worm was not found in the fish later in the summer, and a new infection was obtained early in the fall to mature the following summer.

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CERCARIA HUTTONI, SP. NOV., A DERMATITIS-PRODUCING SCHISTOSOME LARVA FROM THE MARINE SNAIL, *HAMINOEA ANTILLARUM GUADALUPENSIS* SOWERBY[1]

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During 1949–50 Mr. Robert Hutton of the Marine Laboratory, University of Miami, was engaged in a search for the cause of sea bather's eruption, a dermatitis of unknown origin that occurs sporadically among sea bathers along the lower east coast of Florida. In connection with this study a search was made for snails infected with the larval stages of trematodes of the family Schistosomatidae, the cause of human dermatitis known as "swimmer's itch" in the fresh water lakes of the United States and Canada.

The study began before dermatitis-producing schistosome cercariae had been reported from marine snails, Penner (1950) being the first to do so. He reported the occurrence of a dermatitis-producing schistosome larva, *C. littorinalinae,* from a marine snail, *Littorina planaxis,* off the California coast. Stunkard and Hinchliffe (1951, 1952) attributed a severe dermatitis in Narragansett Bay, Rhode Island, to *Cercaria variglandis* Miller and Northrup, 1926, from *Nassa obsoleta.* A similar and probably identical dermatitis of clam diggers in Long Island Sound, New York, was reported by Orris and Combes (1952). The most recent report of an experimental dermatitis being identified with cercariae from marine snails is that of Chu (1952) in Hawaii. This cercaria, from *Littorina pintado,* was tentatively identified as *C. littorinalinae* Penner, 1950.

Hutton (1952) found two species of schistosome cercariae in marine snails in the Miami area. These were figured as *Cercaria H* from *Nassarius vibex* Say and *Cercaria J* from *Haminoea antillarum guadalupensis* Sowerby. He did not make a morphological study of these cercariae. The single infected specimen of *N. vibex* died before he could carry out exposures on human volunteers. *Cercaria J* from *H. a. guadalupensis* was used by Hutton and later by Leigh (1952) to produce a dermatitis clinically similar to sea bather's eruption.

What is undoubtedly Hutton's *Cercaria H* has been recovered from *N. vibex* during the summer of 1952. This cercaria is a new species which produces as severe a dermatitis as that caused by the cercaria from *H. a. guadalupensis.*

The purpose of this paper is to describe Hutton's (1952) *Cercaria J* as a new species of schistosome cercaria, *Cercaria huttoni,* from the marine gastropod, *Haminoea antillarum guadalupensis.*

Cercaria huttoni n. sp.

(Figs. 1–6)

Specific Diagnosis: Apharyngeal, brevifurcate, schistosome cercaria with pigmented eyespots. Body, tail-stem and furcae are uniformly equipped with minute spines. The tail is terminally attached and dorsal and ventral finfolds extend the full length of the furcae.

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[1] This investigation was supported (in part) by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

Measurements[2] in microns of 30 formalin-fixed specimens: body length 135–164, mean 152 ± 1.4; body width 48–64, mean 53 ± 0.7; length of anterior organ 54–70, mean 63 ± 0.6; length of tail-stem 140–166, mean 154 ± 1.2; width of tail-stem 16–22, mean 19 ± 0.3; length of furcae 72–86, mean 79 ± 0.8; width of furcae 6–13, mean 10 ± 0.4; diameter of ventral sucker 17–22, mean 20 ± 0.3; distance from center of ventral sucker to posterior end of body, 35–51, mean 44 ± 1.0.

Three pairs of large penetration glands extend from the posterior end of the body to the level of the eye spots. These glands overlap considerably and pass their secretions anteriorly through three pairs of conspicuous ducts. The first pair of glands is oxyphilic; the second and extensive third pair are basophilic. The third pair of glands extends from the posterior end of the body where ventral lobes project, to a position dorsal and anterior to the middle pair of glands.

The penetration ducts curve sharply in a ventral direction just anterior to the eye spots and then turn dorsally to become greatly attenuated before entering the ventral side of the anterior organ. No penetration spines were observed. A pair of small escape glands, strongly eosinophilic, lies anterior to the penetration glands in cercariae sectioned within the snail host. The digestive system is a narrow tube extending posteriorly to the level of the eye spots where it divides into two short ceca. The excretory system consists of five pairs of flame cells, $2[(2)+(2+1)]$, four pairs in the body and one pair in the anterior part of the tail. Of the four body pairs two are preacetabular and two are postacetabular. Anterior and posterior collecting tubules unite on each side at the level of the acetabulum to form the arms of a Y-shaped excretory bladder. Each arm has two ciliated areas near the junction of the collecting tubules. The large powerful anterior organ often contains a head gland, but it is not uniformly present.

Host: *Haminoea antillarum guadalupensis* Sowerby.

Locality: Grassy shallow areas near Virginia Key, Miami, Florida.

The literature reveals the existence of at least 31 species of schistosome cercariae. Cort (1950), in a review of schistosome dermatitis, listed 18 species which have been implicated in this problem. Brooks (1946) reported 2 more species of dermatitis-producing cercariae from Wisconsin. Two marine species have been implicated by Penner (1950) and Stunkard and Hinchliffe (1951).

C. huttoni is readily distinguished from all other species of schistosome cercariae by the number of flame cells and penetration glands. It is the only species with eye spots to possess 5 pairs of flame cells and three pairs of penetration glands. *Cercaria Indica* XLVII Sewell, 1922, possesses 5 pairs of flame cells, but has 5 pairs of penetration glands. The other species have either six, seven or eight pairs of flame cells and two, five or six pairs of penetration glands.

DISCUSSION

Cercariae emerge in the early evening hours and most of them are dead in sea water at the end of a twenty-four hour period. They characteristically lie in contact with the surface film and are dislodged from it only after considerable agitation. They are inactive while attached to the surface film except for occasional slight twitchings of the tail. Body and tail are greatly contracted while at the surface film, in contrast with their extended state while swimming or crawling on the bottom.

Swimming consists of rapid sidewise motions of body and tail. The ventral sucker and base of the tail stem appear to serve as fixed points between which the body and tail stem oscillate. Locomotion is slow but, since the tail is normally pointed upwards, movement brings the cercariae in contact with the surface film where they collect on the side closest to a source of light.

The snail host, *H. a. guadalupensis*, is a non-operculate snail which inhabits

[2] Talbot (1936) method was used in fixing cercariae, substituting sea water as a diluent. Extremes and standard error of the mean indicate the character of the variation.

grassy vegetation in the shallow water of Biscayne Bay, Florida. *C. huttoni* has been found only on the north side of Virginia Key. This area consists of sand flats which are exposed at low tide and is frequented by numerous great blue herons, turnstones, dowitchers and gulls. Collections of *Haminoea* in other areas of Biscayne Bay have been negative for any kind of cercariae. The incidence of infection is low. Of 1259 snails collected from the north side of Virginia Key from August 1951 to February 1953 only ten were infected. The isolation technique was used to determine evidence of infection. Subsequent crushing of snails revealed no further infections.

Although the relationship of schistosome cercariae to sea bather's eruption has not been determined, the lesions produced by cercariae from *N. vibex* and *Cercaria huttoni* appear to be identical to those seen in that condition. The intensity and duration of the experimental dermatitis are similar to descriptions of sea bather's eruption (Sams, 1949) and swimmer's itch (Cort, 1950).

The following description of the dermatitis is based on several exposures of the author to *C. huttoni,* either by resting the arm on the surface film of water containing cercariae or by dropping them on gauze in contact with the arm. The first signs of penetration are noticed in about 12 minutes, and from 15 to 25 minutes the stinging sensations are intense. After 45 minutes the points of penetration are visible as small macules approximately one millimeter in diameter. After four hours the macules have increased from 2 to 5 millimeters in diameter. During the second and third days papules, which replace the macules, are especially prominent and erythema occurs over and around the entire area of penetration. Itching is sporadic, sometimes intense, and is stimulated by contact, rubbing, or sweating. On the fourth day the macules are reduced and itching is mild and sporadic. By the seventh day only minute, dark red spots remain.

Hutton (1952) regarded marine cercariae as the probable cause of sea bather's eruption in spite of a striking unexplained difference between cercarial dermatitis and sea bather's eruption. This concerns the peculiar localization of the latter on areas covered by the bathing suit; swimmer's itch occurs predominantly on exposed areas of the body. Hutton suggested that this localization may be due to the adverse effect of more rapid drying of salt water on the exposed surface of the body. However, Stunkard and Hinchliffe (1952) found no such localization in connection with a marine cercarial dermatitis in Narragansett Bay, Rhode Island.

Hutton (1952) summarized records on sea bather's eruption from 1936 to 1950 and found that cases occurred from March to August with the majority falling in May and July, indicating that this is a summer involvement. Stunkard and Hinchliffe (1952), in the only report of a marine cercarial dermatitis involving many cases, found that the major infection of *Nassa obsoleta* takes place in the late fall and that liberation of cercariae occurs during the colder months of the year. In the present study, infected *Haminoea* taken in October and February were producing numerous cercariae which produced an experimental dermatitis.

Sams (1949) gave a thorough account of the clinical aspects of sea bather's eruption and concluded that it should not be confused with swimmer's itch, which at that time was regarded as strictly a fresh water cercarial dermatitis. His histological examinations revealed no foreign bodies or parasites in lesions excised from patients. However, none of his material was taken earlier than 24 hours

following exposure. Although Brackett (1940) was unable to demonstrate cercariae in the skin 29 hours following exposure, Macfarlane (1949) found that there was only a mild histolysis of the cercariae in the skin at the end of 2 days. Conclusive association of schistosome cercariae with sea bather's eruption will rest on examination of sections of skin biopsies from natural cases taken earlier than the 24 hour sections now available.

SUMMARY

A dermatitis-producing schistosome cercaria, *Cercaria huttoni,* n. sp. is described from *Haminoea antillarum guadalupensis* Sowerby, a marine non-operculate snail which inhabits the grassy vegetation in the shallow water of Biscayne Bay, Florida. Experimental production of a dermatitis in human volunteers is reported. The relationship of this cercaria to sea bather's eruption is not determined.

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EXPLANATION OF FIGURES

PLATE I

FIG. 1. Flame cell system of *Cercaria huttoni.*

FIG. 2. Photograph of *C. huttoni.*

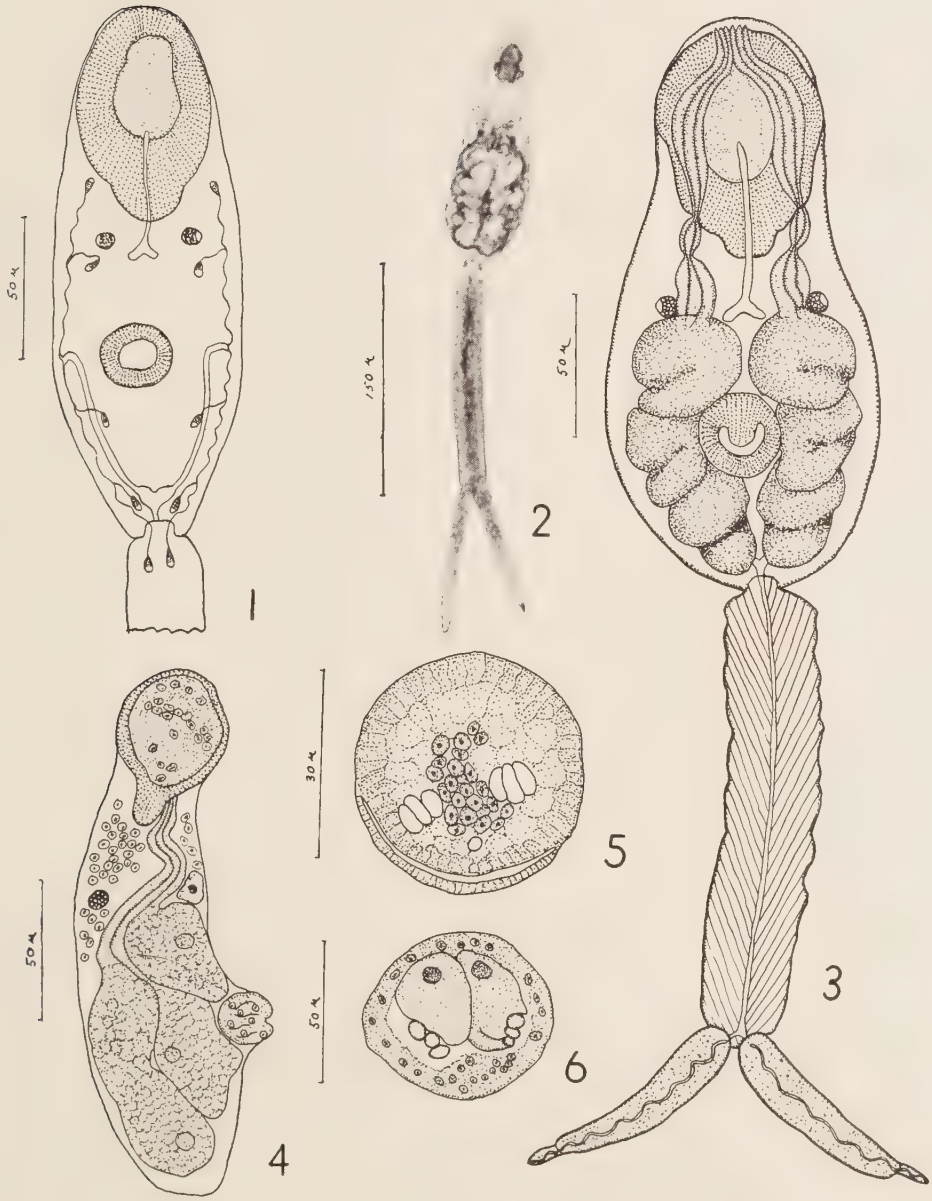
FIG. 3. *C. huttoni,* ventral view, drawn from life.

FIG. 4. Saggittal section of *C. huttoni,* showing arrangement of penetration glands.

FIG. 5. Cross section of *C. huttoni* at level of the anterior organ, showing the three pairs of penetration ducts.

FIG. 6. Cross section of *C. huttoni* immediately anterior to first pair of penetration glands.

PLATE I



SCIOMYZID LARVAE (DIPTERA) THAT FEED ON SNAILS[1]

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This progress report is published to appeal for materials to extend the present investigation and to release immediately some observations that may have practical applications of considerable value. It is based upon investigations conducted during the summers of 1950, 1951 and 1952 in the Matanuska Valley and the lower Yukon Valley in Alaska, in Oakland County, Michigan during the summer of 1949; and at Delaware, Ohio during the past two academic years. I am indebted to Mr. George C. Steyskal, of Grosse Ile, Michigan, for identification of the flies and to Mr. Dwight W. Taylor, Museum of Zoology, University of Michigan, and Dr. Harald A. Rehder, U. S. National Museum, for identification of the snails here discussed.

I have reared SCIOMYZIDAE (TETANOCERIDAE or TETANOCERATIDAE) of six species, *Dictya expansa* Steyskal, *Hedroneura rufa* Panzer, *Sciomyza dryomyzina* Zetterstedt, *Sepedon fuscipennis* Loew, *Tetanocera ferruginea* Fallén and *T. rotundicornis* Loew, from larvae that fed on living or freshly killed snails. Rearings of species in four additional genera, *Colobaea* and *Ctenulus* (Lundbeck, 1923), *Ditaenia* (Schmitz, 1917), and *Salticella* (Mercier, 1921; Blair, 1933), from snails are recorded in the European literature. According to unpublished information received from Mr. Steyskal, a specimen of *Pteromicra* was once bred from a snail in Canada.

Since the ten genera listed above represent three subfamilies, including the two (SCIOMYZINAE and TETANOCERINAE) that together embrace all Nearctic species and more than 90 per cent of the known SCIOMYZIDAE of the world, it would appear that the snail eating habit is very widespread among larvae of this family. Adult swale flies (or marsh flies) are commonly caught only in habitats of aquatic or terrestrial snails, and I find in the literature not one well verified record of sciomyzid larvae feeding on anything but snails.

Statements of various contemporary authors that sciomyzid larvae appear to be catholic in their feeding habits (e.g. Colyer and Hammond, 1951) may result from speculative or impressionistic remarks of several early writers and from an instance of obviously faulty logic. Dufour (1849), citing no proof and presumably having none, wrote: "Je me suis assuré que cette larve se nourrit des immondices et mucosités qui se trouvent au milieu de ces plantes aquatiques." Lundbeck (1923) reared *Ditaenia cinerella* ". . . only from the puparia without knowing the larvae and thus not being able to decide anything as regards their feeding habits." Nevertheless, in contending against Schmitz's evidence (1917) that these larvae may feed on snails, he unfortunately declared, ". . . as I have the same species from flood refuse it is certainly no snail feeder, its presence in the shell being quite occasional." Since all sciomyzid larvae I observed feeding on snails leave the shells before pupation (and since most parasitoid and predaceous larvae of other dipter-

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[1] Contribution from the Arctic Health Research Center, U. S. Public Health Service, Anchorage, Alaska; the Cranbrook Institute of Science, Bloomfield Hills, Michigan; and the Department of Zoology, Ohio Wesleyan University.

ous families similarly leave the remains of their victims), the finding of free-floating puparia cannot be accepted as evidence that larvae of that species do not feed on snails.

If great numbers of snails are eaten by sciomyzid larvae, these insects may serve as subsequent hosts of helminth and protozoan parasites the early stages of which occur in snails. Investigators attempting to work out life-cycles of such snail parasites should not overlook sciomyzid larvae, pupae and adults in their search for the next host and next stage of the parasite.

With the exception of *Tetanocera rotundicornis,* known larvae of the four tetanocerine genera *Dictya, Hedroneura, Sepedon* and *Tetanocera* are free-living and aquatic. Although I have found no reference to their snail eating habits in the literature, all that I have observed attack aquatic snails, kill them quickly and consume most or all of the soft parts in a few hours. These larvae frequently enter the shells and consume the remains of freshly killed and partly eaten snails, but the precision and speed with which they attack and kill living snails (even larger than themselves) provides abundant evidence that they are primarily predators rather than scavengers. Hungry larvae in second and third instars (perhaps younger larvae also) often attach to the shells of moving snails and allow themselves to be carried along while they probe about until they can find and sink their mouth-hooks into the exposed soft parts. During the struggles that follow, as snails either attach the tips of their feet and throw themselves and the larvae violently about or retract and pull their adversaries deeply and forcefully into the shells, the larvae are very seldom forced to drop off. Since snails usually cease to struggle in a minute or less, it seems that the larvae must either inject venom into their prey or quickly destroy some major blood vessel or nerve center. Mature specimens of *Helisoma subcrenatum* (Carpenter) yield conspicuous amounts of mucus and blood during and after their death struggles.

Snails are not fed upon merely as a last resort during starvation conditions in laboratory rearing bowls. The evidence of proficiency given above would indicate either previous experience with the process of snail predation or well developed instinctive behavior. Furthermore, I once found a third instar larva of *Sepedon fuscipennis* eating a small *Lymnaea emarginata* in nature (July 23, 1952, at Fire Lake, near Anchorage, Alaska). This larva, prodded until it withdrew from the shell so that I could examine the remaining snail tissues (which proved to be strictly fresh), evidently returned during the night and consumed all remaining soft parts. When picked up on a camel's hair brush the following afternoon and lowered, head first, toward the aperture of a living but retracted *Lymnaea palustris,* it attacked immediately—while its posterior end was still supported on the brush. That evening the larva was still busily feeding and well inside the shell. By morning it had withdrawn, but the snail shell was thoroughly cleaned.

Each larva that lived for a week or more between capture and pupation destroyed several healthy, active snails (some of them immature) which I selected from three or four genera. Larvae of *Dictya expansa* killed and devoured specimens of *Physa* cf. *gyrina* Say, *Lymnaea obrussa* Say and *Zonitoides arboreus* (Say). *Sepedon fuscipennis* larvae destroyed individuals of *Lymnaea palustris* (Müller), *L. emarginata* Say, *L. stagnalis* (Linné), *Helisoma* sp. and *Oxyloma decampi gouldi* Pilsbry. In addition to *L. palustris, Helisoma* sp. and *O. d. gouldi,*

larvae of *Tetanocera ferruginea* killed specimens of *Gyraulus deflectus borealis* (Westerlund).

The possibility that these relatively unselective snail predators may prove efficient in the biological control of undesirable snails merits thorough investigation. Since adult swale flies are inoffensive and apparently harmless, there would seem to be no great risk in voluntarily extending their ranges. Although most of the SCIOMYZIDAE occur only in Nearctic and Palearctic regions, species in some genera (e.g. *Sepedon* and *Protodictya*) thrive in tropical climates where man's trematode diseases are most prevalent.

Immature stages of *Tetanocera rotundicornis* and *Sciomyza dryomyzina* live under conditions quite different from those of aquatic tetanocerine larvae. On August 14, 1952, more than 300 *O. d. gouldi* were collected on *Equisetum,* willow and other vegetation growing on low, swampy ground one-half mile north of Matamuska Junction, Alaska. No dead or inactive snails were noticed during their collection nor their transfer a few days later to larger containers, and no dipterous larvae were observed at either time. The snails were not examined carefully, however. On August 22, a few snails were dead, and the cast pharyngeal skeleton of a fly larva was found within the shell on the partly eaten body of one of them. Seven active larvae and two similar, compact, translucent brown puparia were then discovered in the large finger bowls used as rearing cages. Since neither soil nor vegetation (in fact, nothing except the snails) was placed in the finger bowls, the possibility of accidental introduction of these larvae can be discounted. At least some of the larvae quickly killed and partly consumed succineid snails that were introduced into the small container into which they were then placed. Most of them became listless and ceased feeding, however, and all except one died during the following fortnight. The remaining specimen formed a puparium on September 10 that was larger and distinctly different from the other two. On October 9, when these three puparia had stayed in moist sand at room temperature for several weeks, they were placed in a refrigerator, where they remained at 37 degrees Fahrenheit until November 26. An adult female of *S. dryomyzina* emerged from one of the smaller puparia on December 22, 26 days after being returned to room temperature. On February 18, five months and eight days after pupation, a male of *T. rotundicornis* emerged from the larger puparium. Another male of this species was obtained on June 19, 1951, from a puparium found about two inches below the surface of the moist, sandy soil north of Matanuska Junction on June 12.

Mature larvae of *Tetanocera rotundicornis* are terrestrial predators on snails. They lack hydrofuge, palmate hairs such as those which surround the posterior spiracular discs of *T. ferruginea, T. robusta* Loew and other aquatic tetanocerine larvae, and when they are placed in water their posterior spiracles seem as likely to submerge as any other part.[2] It is evident from the puparia that larvae of *S. dryomyzina* also lack these palmate hairs, and Johannsen (1935) indicated that the same is true of *Poecilographa decora* Loew. Apparently all three species are exceptions to the categorical but frequently repeated declaration of authors that "the sciomyzid larvae are aquatic." It is not improbable that larvae of many species

[2] Presence of hydrofuge hairs on aquatic larvae and their absence from terrestrial larvae in the same genus tends to negate the contention of some zoologists that differences between the species within a common genus are never of adaptive significance.

live in damp soil and that our delay in discovering them is accountable to their relatively concealed positions in nature.

It might be inferred from my failure to notice larvae of *T. rotundicornis* during collection and subsequent transfer of the snails that a more intimate, parasitoid relationship exists between these two animals until the larvae become nearly mature. It is possible, however, that they are predatory throughout larval life, that each larva was within a snail shell, eating the recently killed mollusk, when both were collected, and that the dead snail stayed up on the vegetation only because the shell had been affixed by mucus to the plant before the larva attacked.

The observations on *S. dryomyzina* recorded above and the (equally circumstantial and fragmentary) evidence available concerning all other reared species in the sciomyzine genera *Colobaea, Ctenulus, Ditaenia, Pteromicra* and *Sciomyza,* and in the genus *Salticella* (SALTICELLINAE) suggest that their larvae live within snail shells and feed on snail tissues for several days before killing them. Most of these larvae (including *Sciomyza dubia* Fallén, reported by Oldham, 1912) pupate within snail shells, and Lundbeck (1923) presented evidence that certain of them are quite host specific. Although Vimmer (1925) figured the pharyngeal skeleton of *Sciomyza ventralis* Fallén, it seems that no larva in these two subfamilies has ever been described adequately.

Evidence that sciomyzid larvae of so many species eat snails raises some interesting academic points in addition to its practical implications to parasitologists. It suggests that the SCIOMYZIDAE, long considered by taxonomists as a natural group morphologically, may be integrated biologically by the common food preferences of their larvae. Like certain other better known groups, sciomyzid larvae illustrate various degrees of intimacy of association with the animals upon which they feed (from free-living, predatory larvae to apparently obligate parasitoids), possibly corresponding to successive stages in the evolution of this relationship. It may at first glance appear paradoxical that at least some larvae of the TETANOCERINAE, adults of which are usually regarded as the most advanced of the SCIOMYZIDAE morphologically, have retained the most primitive larval feeding methods.

The large air bubbles nearly always present in intestines of aquatic sciomyzid larvae and considered by some writers as flotation devices probably serve some other function. They are not routinely present in aquatic larvae of other families (e.g. TIPULIDAE and STRATIOMYIDAE) that are similarly oriented to the surface film, but they occur consistently in terrestrial as well as aquatic sciomyzid larvae. They result from voluntary ingestion of air, usually at intervals during the feeding process. Feeding larvae that have crawled far into large snail shells often have their posterior spiracles isolated from contact with the air for considerable periods of time. I have no evidence, however, either that the relatively small amount of oxygen contained in these intestinal bubbles can be used for respiration in such emergency situations or that it is associated with processes of digestion. Because of the habit of ingesting air and other biological and structural characteristics reported by Bhatia and Keilin (1937), it seems highly probable that their unidentified snail-eating larvae are terrestrial SCIOMYZIDAE.

Adults of *Limnia boscii sparsa* Loew, captured in nature, mated freely in lamp chimney breeding cages and laid viable eggs. Wild caught females of *Tetanocera*

unicolor Loew attached their eggs to damp sphagnum moss at the bottom of the container. Newly hatched larvae of *T. unicolor* ate pieces of freshly killed *Helisoma subcrenatum* (Carpenter), frequently interrupting the process to swallow air, but they died while still in the first larval instar.

It may be deduced from available data that Sciomyzidae pass the winter in both immature and adult states. A larva of *T. ferruginea,* collected August 14 at Fire Lake near Anchorage, Alaska, pupated September 3, and the adult emerged (in a heated room) December 22. Larvae were not found in June and July, but puparia of this species taken in the same locality June 26 and July 3 yielded adult flies a few days after collection. These records and the data presented above on *T. rotundicornis* and *S. dryomyzina* clearly indicate that these three species normally pass the winter as pupae.

On the other hand, larvae of *S. fuscipennis* and *H. rufa,* collected throughout the summer in the Matanuska Valley, Alaska, pupated during June, July and August, and adult flies emerged seven to nine and one-half days after pupation. Specimens of *D. expansa,* reared from larvae collected in May and June at Delaware, Ohio, passed through the pupa stage in about three weeks. The short pupal periods observed and the fact that adults of some species are found very early in spring and even during mild weather in midwinter would suggest hibernation of adult flies.

A minute, flightless diapriid, described by Muesebeck (1949) as *Phaenopria popei,* was reared in numbers of 17 to 30 from each parasitized puparium of *S. fuscipennis* in southern Michigan but was not encountered in Alaska.

Keys, descriptions and figures of immature stages of Nearctic Sciomyzidae will be presented in a forthcoming paper. The free-living, aquatic larvae resemble tipulid larvae in bearing large, terminal posterior spiracular discs surrounded by four, six or eight fleshy lobes or papillae. Their soft, cylindrical bodies, encircled by several bands of rounded tubercles, taper at the ends (anterior, especially) and have no sclerotized head capsules. The floating puparia formed by aquatic tetanocerine larvae have arched ventral sides, more flattened dorsal sides and dorsally curved posterior ends, some of which taper to long, slender tips. Larvae of *T. rotundicornis* have only four, very short and blunt papillae along the ventral edge of the spiracular disc, and the bands of rounded tubercles are lacking. Puparia of *T. rotundicornis* and *S. dryomyzina* are compact and barrel-shaped, similarly arched dorsally and ventrally, and with posterior ends abruptly tapered and scarcely if at all dorsally curved.

Diptera of various families in addition to the Sciomyzidae may act as agents of biological control of snails and as subsequent hosts of parasites found in snails. I have reared Sarcophagidae from puparia found in snail shells (material supplied by Mr. John B. Villella, parasitologist at the University of Michigan) and observed that certain tabanid larvae are predatory on snails under laboratory conditions. Diptera of the families Anthomyidae, Borboridae, Calliphoridae, Chironomidae, Dryomyzidae, Ephydridae, Muscidae, Phoridae, Psychodidae, Sarcophagidae, Sepsidae and Tabanidae have been reared from snails by other investigators, and much more is known about some of them than about any sciomyzid larva. Larvae in some of these families are ineffective agents of control because they feed only on secreted mucus or on dead snails.

Materials for the extension of this study are solicited. Free-living aquatic larvae of SCIOMYZIDAE are found at the borders of ponds, lakes and streams, especially in the floating debris that accumulates against stumps, fallen branches and other obstructions. In addition to them, all dipterous larvae and puparia found within snail shells are of considerable interest to me. While preserved larvae will be of value eventually, there is at present no way to identify them. Living larvae and pupae or reared adults (preferably pinned) associated with their puparia are considerably more valuable, at least until a key to immature stages can be formulated. Living material can be shipped in slightly damp (not wet) sphagnum moss packed loosely in a container with openings to admit fresh air. Biological data and preserved specimens of host snails are very desirable. One or two living snails should be included with each group of living larvae. I will gladly pay packing charges and air mail postage.

SUMMARY

1. SCIOMYZIDAE of six species in five genera were reared from larvae that fed on living or freshly killed snails. Since flies representing five additional genera have been reared from snails by others, larvae in ten genera distributed among three subfamilies of SCIOMYZIDAE apparently feed on snails.

2. Because snail eating appears to be widespread among larvae of this family, the SCIOMYZIDAE possibly serve as subsequent hosts of helminth and/or protozoan parasites found in snails. Students of the life-cycles of these snail parasites might well examine members of the SCIOMYZIDAE in their search for the next host.

3. Larvae of the tetanocerine genera *Dictya, Hedroneura, Sepedon* and *Tetanocera* attack snails of at least nine species in six genera (mostly aquatic), kill them quickly and consume most of the soft parts in a few hours.

4. It is suggested that these relatively unselective snail predators may prove useful in the control of undesirable snails.

5. Sciomyzine and salticelline larvae apparently live within snail shells and feed on snail tissues for several days before killing the mollusks.

6. Some sciomyzid larvae lack certain modifications of their aquatic relatives and live in damp soil. Since the intestinal air bubbles considered by some writers as flotation devices occur in terrestrial as well as aquatic sciomyzid larvae, they probably serve some other function.

7. Mating and oviposition of SCIOMYZIDAE in laboratory breeding cages and activities of newly hatched larvae are discussed briefly.

8. Data are presented indicating that SCIOMYZIDAE pass the winter in both immature and adult states.

9. Each parasitized puparium of *S. fuscipennis* yielded many (up to thirty) small HYMENOPTERA.

10. Sciomyzid larvae and puparia are described briefly.

11. Brief reference is made to tabanid larvae accepting snails as food and to SARCOPHAGIDAE reared from puparia in snail shells by the author, and to DIPTERA of these and other families associated with snails by other investigators.

12. To extend both biological and morphological aspects of this study, living or reared DIPTERA that feed on snails are solicited.

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EFFECT OF 2–AMINO–5–NITROTHIAZOLE (ENHEPTIN) AND OTHER DRUGS ON *TRICHOMONAS GALLINAE* INFECTION IN THE DOMESTIC PIGEON

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There are four species of trichomonads which are described as pathogenic: *Trichomonas vaginalis* in man; *T. foetus* in cattle; *T. gallinarum* in gallinaceous birds, especially in turkeys; and *T. gallinae* in various birds, particularly the columbids. A closely related flagellate, *Histomonas meleagridis,* is the cause of enterohepatitis (blackhead) in turkeys. The treatment of infections caused by these organisms still leaves much to be desired. Therapeutic attempts in acute trichomoniasis due to *T. gallinae* have met with failure. In the chronic carrier of *T. gallinae* a great variety of agents has been tried. Copper sulfate has been the most widely used and some success has attended its administration in the carrier's drinking water (Jaquette, 1948a). In 1949 Waletzky, *et al* introduced a new compound, 2-amino-5-nitrothiazole (Enheptin), in the treatment of these flagellates. It showed promise against the enterohepatitis-producing *H. meleagridis* in turkeys (McGregor, 1952; Swales, 1952).

The Lederle Laboratories of the American Cyanamid Company and the Cutter Laboratories, Berkeley sent the author several drugs to be tried against pigeon trichomoniasis due to *T. gallinae,* among which was 2-amino-5-nitrothiazole (Enheptin, hereinafter designated as ANT) from Lederle. It is the purpose of this paper to report the effects of these drugs on chronic and acute infections with *Trichomonas gallinae* in the domestic pigeon (*Columba livia*).

MATERIAL AND METHODS

Pigeons. The birds were of two sources: 1) 2–8 month old homers from the author's *Trichomonas*-free loft (Stabler and Engley, 1946), which had not been exposed to *T. gallinae* for twelve years; and 2) adult pigeons, all chronic carriers of *T. gallinae,* from the stock of a local commercial raiser. These latter were a breed-mixture of white king, carneau, and homer. One white king was also used. The average weights were: homers 351.5 gm.; commercial birds 541.0 gm.; the white king 503.0 gm.

Drugs. Except in the case of the Cutter preparation (PSSS), which was given in terms of USP units, the agents were used in various amounts per kg. of body weight. To simplify administration the substances were capsuled in various milligram amounts. The schedule of dosages for ANT is given in Table 1.

The Cutter preparation was a combination of crystalline potassium penicillin plus sulfadiazine, sulfamerazine, and sulfamethazine. Aureomycin, nitrophenide (Megasul), and ANT were the Lederle products tested.

Parasite. The parasite was *Trichomonas gallinae.* Three varieties were used: 1) the author's extremely virulent strain of *T. gallinae,* known as the Jones' Barn strain, and 2) a strain derived from a cankerous falcon, known as the Mirza strain,

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TABLE 1.—*Effect of various dosages of ANT (2-amino-5-nitrothiazole) in 50 pigeons naturally or artificially infected with* T. gallinae.

Dosage		Number of birds	Total mg. received per bird	Results
Mg. per day	Mg. per kg.			
5	9	2 homers	35	2 homers, 1 comm. became neg.;
	14	2 comm.	35	1 comm. remained pos.
	28	2 homers	40	
	28	1 homer	50	
	28	1 homer	60	
10	28	23 homers	70	all became permanently neg.
	18	7 comm.	70	
	28	2 homers	80	
	28	1 homer	90	
20	40	1 white king*	80	became neg.
25	45	2 comm.	175	became neg.
50	90	2 comm.	1 got 450 1 got 500	both died, at total amounts indicated; neg. at death
75	135	2 comm.	1 got 300 1 got 375	both died, at total amounts indicated; neg. at death
100	280	2 homers	700	1 died, 1 barely survived; neither showed *T. gallinae*

* remained positive after a previous course of 10 mg. (20 mg./kg.) ANT daily for 10 days.

these two being previously referred to as strains I and V (Stabler, 1948); and 3) the trichomonads occurring in the chronically infected commercial pigeons.

Untreated controls were run against the treated Jones' Barn and Mirza strain pigeons. No birds from the commercial source were known to die of trichomoniasis, although all were positive. At the present writing (23 July 1952) the Jones' Barn strain has been placed in 81 consecutive homers serving as control. Of these, five have survived and 76 (93.8%) have died of trichomoniasis, death occurring at 8.4 days (av.). Of three Mirza strain controls: two died of trichomoniasis, one survived its infection.

OBSERVATIONS

PSSS

Five chronic carrier, commercial pigeons (av. 541.0 gm.) were given this preparation every six hours for four doses. Total amounts ranged from 62,500–1,000,000 USP units per bird. Four remained positive; one (receiving 250,000 units) lost its *T. gallinae* and remained negative. These birds were housed individually, and as it is known that such pigeons tend to lose their trichomonads (Jaquette, 1948b), any possible curative effect of PSSS here must be viewed with caution.

Each of two *T. gallinae*-free homers (av. 351.5 gm.) now received 250,000 units of PSSS prophylactically in four doses at six hour intervals. They were then given orally the Jones' Barn strain. Over the next five days each received a total of 937,500 units of PSSS. One died of trichomoniasis at eight days, one at nine. This drug, then, appears to have little effect against either chronic or acute infections with *T. gallinae*.

Aureomycin

Two *T. gallinae*-free homers received single daily doses of 140 mg./kg. of aureomycin HCl prophylactically for three days. Each was then given Jones' Barn *T. gallinae*, followed by 140 mg./kg. of aureomycin daily until death. One died at five, the other at six days following infection. Aureomycin did not protect these birds from virulent *T. gallinae*.

Nitrophenide

One hundred twelve mg./kg. daily of this drug were given prophylactically for three days to each of two *T. gallinae*-free homers. Each then received Jones' Barn *Trichomonas,* followed by 140 mg./kg. of nitrophenide daily until death. One died at four days, the other at five days. Nitrophenide appears ineffective against virulent *T. gallinae.*

ANT

This drug, 2-amino-5-nitrothiazole (Enheptin), was a dramatic success. It is, furthermore, the only substance yet tried which will control infections with virulent strains of *Trichomonas gallinae.* Of 28 birds infected with the Jones' Barn strain and treated with ANT no bird died. In the 81 untreated controls, 76 succumbed. Moreover, as administered in these experiments the minimal trichomonacidal dose was well below the toxic level for pigeons.

Dosages (Table 1). In what proved to be toxic amounts, ANT was given to six pigeons as follows: each of two infected commercial birds received 90 mg./kg. daily, one receiving 450 mg., the other 500 mg.; two more positive commercial birds received 135 mg./kg. daily up to 300 mg. and 375 mg.; and two *Trichomonas*-free homers each received 280 mg./kg. daily for three days. The homers were then given the Jones' Barn strain, followed by four more daily doses of ANT at 280 mg./kg. up to a total of 700 mg. each. The four commercial birds became negative for *T. gallinae,* but all died. The homers at no time appeared positive, one dying and the other barely surviving. Prior to death these birds experienced difficulty in standing and showed twisting movements of the head.

In contrast to this it was found that, although probably on the borderline of effectiveness, a dosage of only 14 mg./kg. daily was sufficient to rid some birds of *T. gallinae.* Treated at this dosage for a week, three (2 homers, 1 commercial) of four birds became permanently negative, though one (commercial bird) remained positive for *T. gallinae.* Fifty-six mg./kg. and 70 mg./kg. daily doses for seven days were tolerated without ill effect in any bird. Although these amounts completely eradicated the *Trichomonas,* they appeared to be in excess of the minimal trichomonacidal dose for birds of the homer weight group (351.5 gm. av.).

Twenty-eight mg./kg. daily for seven days seemed the optimal dosage for homers. Not all birds became negative after the same amounts of ANT, however, as will be indicated. That a 20 mg./kg. daily dose may not be enough to rid some larger birds of *T. gallinae* is suggested by the failure of this dose administered for five days to clear the white king (503.0 gm.) of its trichomonads. A subsequent treatment with 40 mg./kg. daily for four days, however, removed the trichomonads.

All 37 birds receiving 10 mg. ANT daily became permanently free of *T. gallinae* (Table 1). Seven of these were the larger commercial pigeons; the remaining 30 were homers. Of interest is the fact that 25 in this homer series were birds harboring the lethal Jones' Barn *T. gallinae.* The other five homers received the virulent Mirza strain. Not a single ANT-treated bird succumbed to *T. gallinae.*

From these data it appears that powdered ANT by mouth at the dosages indicated is highly efficient as a trichomonacidal agent, with respect to *T. gallinae* in the pigeon; that its maximal therapeutic effectiveness appears to be considerably below any toxic effect, thus allowing a satisfactory margin of safety; that in larger amounts it may be toxic, actually killing the recipient.

Table 1 indicates that ANT was administered up to various total amounts per bird, though the daily doses may have remained constant. The majority (30 birds) received 28 mg./kg. daily for seven days, irrespective of when the birds appeared to become negative. Daily examinations were made on a series of treated birds in order to determine how soon they would become negative following commencement of medication with ANT (Table 2). Failure to recover *T. gallinae* from the fluids of a pigeon's mouth and crop is presumptive evidence that the bird is free of the organism. It is seen from Table 2 that, with the exception of one bird which remained positive, all in this series were free of *T. gallinae* by the eighth day. Also, 13 of the 33 pigeons appeared negative 24 hours after the first treatment.

TABLE 2.—*Days following treatment with ANT (2-amino-5-nitrothiazole) on which pigeons receiving daily examinations appeared negative for* T. gallinae.

Dosage		Number of birds	Day on which birds appeared negative								Remained positive
Mg. per day	Mg. per kg.		1	2	3	4	5	6	7	8	
5	9	1 comm.									1
	14	2 homers				1	1				
10	18	5 comm.	2	2	1						
	28	25 homers	11	3	2	3	3	2		1	

As indicated, pigeons free of *T. gallinae* may be infected with the highly virulent strains of this parasite and then be cleared of the infection by the administration of ANT. In those birds which had developed sufficient immunity, reinfection with the most virulent strains seldom produced more than mild, transitory pathology, with nearly 100 per cent survival. In order to determine whether or not the virulent *T. gallinae* re-inoculated into these immune birds was still capable of producing disease, the trichomonads from three such birds were given to two birds each. The donor birds had lived with their reinfections for 42 days. Of these six recipients, all died of typical trichomoniasis. Whatever the mechanism of immunity, it apparently does not appreciably reduce the virulence of the resident trichomonads.

One bird (white king, 503.0 gm.) was not cleared of its virulent *T. gallinae* after a total of 100 mg. of ANT given in 10 mg. daily amounts. There was no sign of pathology. It was wondered whether or not the drug, although not eliminating the parasite from this bird at this dosage, had so affected the flagellates physiologically that they were no longer capable of producing lesions. Consequently, the trichomonads from this donor, which had lived with its virulent *T. gallinae* for ten days and to which had been given 100 mg. of ANT during that time, were now given to four *Trichomonas*-free homers. All became positive. Two of these were then treated with ANT and cleared of their infections. Two were not treated, and both died of trichomoniasis at six days. In the two untreated birds, then, the virulent *T. gallinae* was still capable of causing death even though it had been under the influence of a sub-trichomonacidal dose of ANT for ten days. Also, the *Trichomonas* was not ANT-fast as it was readily removed from the two recipients which were treated with this drug.

DISCUSSION

ANT (2-amino-5-nitrothiazole, Enheptin) has proven of considerable value in handling turkey blackhead (enterohepatitis due to *Histomonas meleagridis*). Some

birds which are under treatment do die, however. According to Almquist and Johnson (1951) the drug also has been found effective in treating turkey hexamitiasis (infectious catarrhal enteritis due to *Hexamita meleagridis*).

In canker infections in the domestic pigeon due to *Trichomonas gallinae* ANT has shown striking therapeutic effectiveness in the experiments herein reported. All cases of infection, both chronic and acute, were permanently cleared of their trichomonads, except in the case of one bird treated at what was probably the minimal effective daily dose. Furthermore, many birds treated harbored the very virulent strains of the parasite, and had advanced cases of the disease when treatment began. No treated bird was lost due to its infection.

The success of ANT in *T. gallinae* infections makes one wonder what it would do in cases of trichomoniasis due to either *T. vaginalis* in man or *T. foetus* in cattle. Experiments are indicated. The results reported here do not show how effective ANT would be in pigeon trichomoniasis were it given in the food, as is commonly done in the treatment of turkey blackhead. Probably the most practical way to handle trichomoniasis in large pigeon establishments is through the drinking water. Would soluble ANT in potable amounts in the drinking water kill *T. gallinae?* Further work here is urgently needed for, despite the drug's apparent effectiveness, the daily pushing of ANT-bearing gelatin capsules down the throats of large numbers of birds is impractical.

SUMMARY

1. PSSS (crystalline potassium penicillin plus sulfadiazine, sulfamerazine, and sulfamethazine), aureomycin, and nitrophenide (Megasul) appeared therapeutically ineffective against chronic or acute trichomoniasis in the pigeon due to *Trichomonas gallinae*.

2. ANT (2-amino-5-nitrothiazole, Enheptin) was effective against all strains of *T. gallinae* used, including one which has produced a 93.8 per cent mortality in 81 successive control pigeons.

3. Administered in gelatin capsules, a 5 mg. daily dose appeared to be at the minimal effective dose level for birds of the domestic pigeon weight range (350–550 gm.).

4. Seven daily doses of 10 mg. (28 mg./kg. in homers), 20 mg. (40 mg./kg. in a white king), or 25 mg. (45 mg./kg. in large commercial birds) of ANT were tolerated without symptoms and effectively removed *T. gallinae*.

5. Daily doses of 50 mg. (90 mg./kg. in commercial birds), 75 mg. (135 mg./kg. in commercial birds), or 100 mg. (280 mg./kg. in homers), whereas they were completely effective against *T. gallinae*, killed five of six pigeons receiving them.

6. It was indicated that *T. gallinae* does not become ANT-fast, nor does it lose its pathogenicity under 10 day exposure to sub-trichomonacidal ANT in a treated bird.

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STUDIES ON THE LIFE HISTORIES OF *MARITREMA OBSTIPUM* (VAN CLEAVE AND MUELLER, 1932) AND *LEVINSENIELLA AMNICOLAE* N. SP. (TREMATODA: MICROPHALLIDAE)[1]

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I. INTRODUCTION

In Barrett's Pond, near Cold Spring on Hudson, New York, two species of minute xiphidiocercariae were found in *Amnicola pilsbryi* Walker, 1906, and their life histories subsequently traced. These cercariae proved to be larvae of the microphallid trematodes indicated in the title. Both cercariae were experimentally found to penetrate the aquatic isopod, *Asellus communis* Say, 1818, and reach sexual maturity in a variety of birds and mammals. Natural infections of larval and adult stages of both species were also identified.

With few exceptions, members of the family MICROPHALLIDAE are parasitic in aquatic birds, particularly those of the seashore. Several species have been described from fishes, but of these, *Gynaecotyla adunca* has been shown naturally and experimentally to infect various shore birds, and *Microphallus opacus* to reach sexual maturity in the raccoon, opossum, turtles, and snakes. The majority of life history studies previously reported have indicated marine molluscan and crustacean intermediate hosts. The present study is one of the first reports of fresh water snails as intermediate hosts in the family. The brief paper of Carrère (1936) is the only account of brackish water intermediate hosts.

The present specimens of *Maritrema* closely resemble *Maritrema obstipum* (Van Cleave and Mueller, 1932), a species based on a single immature worm from the intestine of the rock bass, *Ambloplites*. Accordingly, comparison was made with the type specimen, obtained from the U. S. National Museum through the courtesy of Dr. E. W. Price. In most of its dimensions it fits into the range of size determined for the present material, but differs in the following features: the greater length, coiling, and smaller diameter of the ductus ejaculatorius; the smaller diameter of the male papilla; and the larger size of the pharynx. These differences are regarded as insufficient to justify recognition of a new species, and the present specimens are therefore tentatively allocated to *Maritrema obstipum*. The species of *Levinseniella* studied proved to be new.

II. HISTORY

Rather complete accounts of several genera of the family MICROPHALLIDAE have been presented recently. *Levinseniella* has been reviewed by Rankin (1939a), *Maritrema* by Rankin (1939b), *Spelotrema* by Cable and Hunninen (1940), *Microphallus* by Stunkard (1951) and general surveys of the family were made by Baer (1943) and Cable and Kuns (1951).

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Baer (1943) raised the subfamily MARITREMATINAE Nicoll, 1909 to familial rank on the basis of the presence of a cirrus sac, and the ring-like distribution of the vitellaria in members of this group. Cable and Kuns (1951) suppressed the family MARITREMATIDAE on grounds of the homogeneity of the MARITREMATINAE and MICROPHALLINAE in both morphology and life history pattern. They argued that microphallid genera show a progressive loss of the cirrus sac and cirrus, together with the acquisition of structures such as the genital papilla and complex genital atrium. Cable and Kuns thus demonstrated natural affinities within the family rather than familial distinctions. The pattern of the vitellaria was regarded as fundamentally similar in both groups, and hence of no value as a familial character.

Rankin (1939a) recognized the following species of *Levinseniella as* valid: *L. brachysoma* (Creplin, 1878) Stiles and Hassall, 1901; *L. propinqua* Jägerskiöld, 1907; *L. pellucida* Jägerskiöld, 1907; *L. howensis* Johnston, 1916; *L. minuta* Price, 1934; *L. indica* Lal, 1936; and *L. carcinidis* Rankin, 1939. Yamaguti (1939) transferred *Spelophallus bucephalae* to *Levinseniella,* and Young (1949) described *L. charadriformis* from shore birds in California.

Rankin (1939b) listed the following valid species of *Maritrema: M. gratiosum* Nicoll, 1907; *M. lepidum* Nicoll, 1907; *M. humile* Nicoll, 1907; *M. linguilla* Jägerskiöld, 1908; *M. nicolli* Travassos, 1921; *M. pulcherrima* Travassos, 1921; *M. sachalinicum* Schumakowitsch, 1932; *M. rhodanicum* Carrère, 1936; *M. sp.* Hadley and Castle, 1938; and *M. ovata* Rankin, 1939. Hadley and Castle (1940) named the species described earlier by them *M. arenaria,* and Yamaguti (1939) described *M. eroliae.* Yamaguti and Nishimura (1944) described the metacercaria of *M. caridinae,* and Shibue (1951) demonstrated that *Cercaria takahashii* Ito and Yokogowa, 1949 is the larva of *M. caridinae.* Rothschild and Clay (1952) stated that *Cercaria oöcysta* Lebour, 1907 proved to be the larva of *Maritrema humile,* hence its correct name is *Maritrema oöcysta* (Lebour, 1907). Rankin (1939b) erected a new genus, *Maritreminoides,* to contain *Maritrema nettae* Gower, 1938 as type, *M. obstipum* (Van Cleave and Mueller, 1932), and *M. medium* (Van Cleave and Mueller, 1932). However, Yamaguti (1939) had erected the genus *Pseudospelotrema* on similar grounds to contain *P. japonicum* (type), *P. uriae,* and *P. cincli.* Baer (1943) suppressed *Maritreminoides* as a synonym of *Pseudospelotrema* on grounds of priority. Hunter and Vernberg (1953) described a new species, *Pseudospelotrema ammospizae.*

Yamaguti (1939) erected the genus *Gynaecotyla* (Syn. *Cornucopula* Rankin, 1939) to contain *Levinseniella squatoralae, L. adunca,* and *L. jägerskiöldi,* whereas Rankin (1939a) had included *L. simillimus* and *L. cruzi* as well. Rankin (1940b) described the life history of *Gynaecotyla nassicola* (Cable and Hunninen, 1938) whose adult stage he had previously described (1939a) and named *Cornucopula sippiwissetensis.*

Young (1938) postulated the life history of a worm which he tentatively identified as *Levinseniella cruzi.* In a later work (1949) he cast doubt on his earlier identification, noting that his specimens differed from *Spelotrema nicolli* only in the species of intermediate hosts utilized. Buttner (1951), in a description of progenesis in various trematodes, demonstrated this phenomenon in *Levinseniella pellucida,* which she found encysted in the crustacean, *Palaemonetes punicus.*

III. MATERIALS AND METHODS

In the present study, both fixed and living material were used. Neutral red, nile blue sulfate, and brilliant cresyl blue were employed as vital stains and Bouin's picric acid fixative was used exclusively. Both phase and ordinary microscopy were used in the examination of living material. Measurements were made on living cercariae, shed by the snail rather than obtained by dissection. Semichon's acetic acid carmine was employed for total mounts and Ehrlich's hematoxylin with an eosin counterstain was found satisfactory for sectioned specimens.

Experiments on excystation were carried on in frog Ringer's solution and physiological saline after attempts with tap and distilled water as well as artificial gastric and pancreatic solutions proved unsuccessful. Excystation of neither species was obtained when mechanical methods alone were used. Best results were secured by keeping cysts in frog Ringer's for seven to ten days at room temperature, permitting bacterial action to break down the cyst wall. When partly broken down, the cyst wall was easily torn with glass needles without damage to the worm, and if allowed to stand for more than a week, the worms excysted spontaneously by rupturing the membrane.

The isopod, *Asellus,* was easily cultured in the laboratory and used in infection experiments. Attempts to raise the snail *Amnicola* were not successful. Laboratory raised white mice and rats, hamsters, chicks, ducklings, canaries, pigeons, and fishes were used. Sunfish, salamanders, and turtles were obtained from various geographical areas.

Successful cultivation of eggs was achieved using continually aerated, filtered pond water. According to the method described by Godman and Willey (1941), young ostracods were kept in the dishes with the embryonating eggs to prevent lethal mold growth.

Initial experiments in the infection of vertebrate hosts were done by dissecting naturally infected isopods and separating the two species of encysted metacercariae, which are easily recognizable. The cysts were counted and forced down the throat of the experimental host with a glass pipette. After the identity of the two species of natural cysts had been established, living infected isopods were fed to the host.

Periodic collections of snails and isopods were made at various times of the year to discover any fluctuations in the incidence of these parasites in their intermediate hosts. These data proved to be of value in identifying the natural intermediate hosts and in detecting differences between the experimental life history and the cycle as it occurs in nature.

Drawings of permanently mounted specimens were made free hand, and with the aid of the camera lucida for major features. Living material was drawn entirely by free-hand.

IV. *Maritrema obstipum* (VAN CLEAVE AND MUELLER, 1932) (Plate I and Text-figure 1)

A. OBSERVATIONS AND EXPERIMENTS

The cercariae are shed primarily in the daytime, the number emerging in 24 hours varying from 0 to 33 per snail under laboratory conditions. The high mortality rate of *Amnicola* in the laboratory suggests that the number of cercariae shed

is probably less than under natural conditions. The cercariae may rest on the bottom of the dish or move about using their two suckers as alternate holdfasts. At intervals of a few minutes they swim upward an inch or so with the body contracted and the tail lashing strenuously. They settle back to the bottom when the tail movements cease, alternately extending and contracting the body. Swimming may be induced in these cercariae by slight movements of the water, but striking the dish with a pencil, or changing the direction or intensity of light has little effect. The length of life of an individual larva is from two to four days, presumably varying with different conditions of temperature, oxygen tension, and other environmental factors.

During August and through September, the incidence of infection in *Amnicola* varied from three to fourteen per cent; the percentage became higher as the weather became colder but decreased slightly after the middle of November. No collections were made in January and February, but in March no infections were found in the few snails collected. Beginning in April, an occasional infection was found and by the end of June a rate of one to two per cent was reached. In July, the number of shedding snails increased markedly, reaching nine per cent in 1951. The total infection was actually somewhat greater as revealed by dissection of snails from which no cercariae had emerged. The number of snails examined per month varied from 28 in March to 1,630 in September.

To discover the second intermediate host, various invertebrates from the infected area were dissected. The finding of metacercarial cysts, of various sizes and stages of development, in *Asellus communis* led to the suspicion that this isopod was an intermediate host. Initial unsuccessful attempts to infect laboratory raised *Asellus* led to experiments with various aquatic insect larvae, salamanders, anuran larvae, snails, turbellarians, crayfish, and amphipods. Attempts to infect these possible hosts also were unsuccessful. When experiments on *Asellus* were repeated, it was apparent that the isopod moved too rapidly and continuously to allow these cercariae to penetrate. Since *Asellus* habitually clings to the undersides of leaves and other objects in nature, pieces of filter paper were placed in the dishes. The isopods soon moved under them and adopted their natural quiescent attitude. In this position, usually ventral side up, *Asellus* remains motionless for one to five minutes, moving only its gill lamellae.

Cercariae were drawn between the gill lamellae by respiratory currents, but actual penetration was not seen. Starting with fifteen cercariae and one isopod per bowl, thirty to sixty minutes was allowed for penetration to take place after which time the number of cercariae always decreased by more than five. Dissection of several isopods seven days later revealed, in each of them, two to seven delicate cysts whose diameters were very slightly smaller than the length of the cercarial body. The stylet had often disappeared and when found, was partially decomposed. The encysted metacercariae were embedded in the connective tissue surrounding the dorsal blood vessel. Later stages of development observed in experimentally infected isopods were found to be identical with the worms discovered earlier in naturally infected isopods. Cysts from experimental infections, 39 days after cercarial penetration of the isopod, were infective to day-old chicks. It is likely that they were infective some time earlier but due to a shortage of experimentally infected isopod hosts, the metacercariae were used only when their cyst

size more nearly approached that of mature natural cysts. Adults obtained from these experimentally produced metacercariae were identical with those from naturally occurring cysts.

The incidence of natural infection in *Asellus* is ninety to one hundred per cent in the months of October, November, and December, but from March through June, the incidence is less than one per cent. The rate increased sharply in early July, but the cysts were usually immature. In early August, more mature cysts were found, their number increasing rapidly in the following month. Thus, the seven to eight weeks from the end of June, when shedding cercariae were first found in numbers, to the middle of August, when the metacercariae became mature, can be taken as an indication of the time required under natural conditions for development in the second intermediate host. This length of time agrees fairly closely with the experimental determination of 39 days, if allowance is made for some increase in rate of development due to higher temperature in the laboratory. The periodicity of infection in isopods was indicative of some migratory or hibernating vertebrate host, and since the members of this family are common in birds, infection experiments were carried out accordingly.

Infections of *Maritrema obstipum* have been produced in white mice, hamsters, white Pekin and mallard ducklings, chicks, pigeons, and canaries. The number of worms recovered from hosts which were fed approximately equal numbers of cysts was found to vary with the duration of the infection. The infection in ducklings lasted as long as 28 days, 24 days in chicks, 5 days in mice, and 2 days in hamsters. Two domestic ducks were found to be naturally infected with more than 100 worms in each. More than 300 gravid specimens were obtained from a single chick 48 hours after it had eaten 150 naturally infected *Asellus*. *Maritrema obstipum* is generally localized in the middle third of the avian small intestine, but worms have been recovered from more anterior and posterior levels as well as the ceca, yolk sac, and cloaca. In mammalian hosts, the worms were always found in the small intestine and never in the cecum.

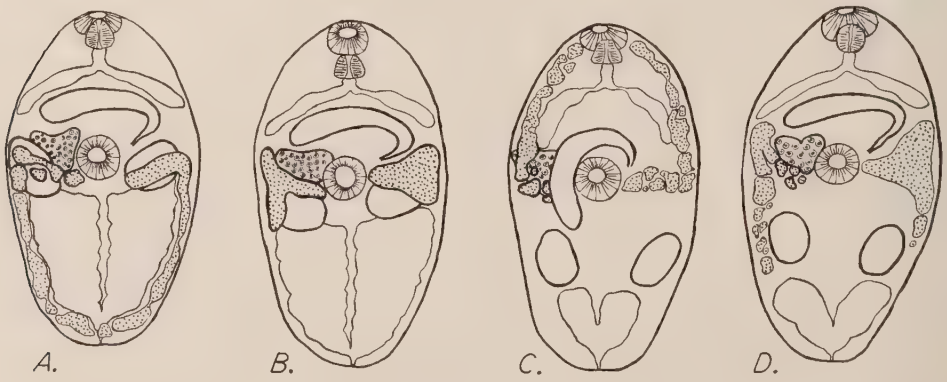
Attempts to infect the turtles, *Chrysemys picta* and *Graptemys pseudogeographica,* and the larval salamander, *Triturus viridescens,* were not successful. In turtles and salamanders dissected within 24 hours after feeding with infected isopods, worms with abortive eggs were sometimes found. Moreover, in metacercariae of *M. obstipum* kept in frog Ringer's and subjected alternately to room temperature and refrigerator temperature (8° to 10° C.) one case of progenesis was observed after 14 days. The worm contained many apparently normal eggs. Infection experiments on the adult salamanders *Eurycea bislineata, Desmognathus fuscus,* and *Triturus viridescens,* the guppy, *Lebistes,* the sunfish, *Lepomis,* the catfish, *Ameiuris,* and the common goldfish were also negative. Autopsies of one specimen of *Blarina brevicauda* and five *Peromyscus leucopus* collected in the infected locality were negative. Museum specimens of several species of water shrews collected in New York, Connecticut, and Michigan, and preserved in alcohol, were obtained through the courtesy of Curator Van Deusen of the American Museum of Natural History, but poor fixation of the internal organs did not allow identification of any intestinal parasites.

B. DESCRIPTION OF STAGES (ALL DIMENSIONS IN MILLIMETERS)

THE ADULT (Plate I, Figs. 3 and 5)

Diagnosis. With the characters of the genus *Maritrema.* Small pyriform to ovoid distomes: covered with minute scale-like spines, more numerous anteriorly. Dimensions in fixed, slightly flattened specimens: body, 0.228–0.517 long by 0.119–0.303 wide; oral sucker, 0.037–0.064 diameter, subterminal, slightly wider than long; acetabulum, 0.031–0.071 in diameter, with short spines anteriorly; prepharynx, very short if present; pharynx, relatively large, 0.017–0.029 long by 0.024–0.037 wide, subspherical, often narrower anteriorly, with flattened anterior and posterior faces; esophagus, short and broad, 0.013–0.044 long, crura, each 0.068–0.130 long, extending laterally along the anterior edge of the cirrus sac almost to the lateral body wall; genital atrium, 0.017–0.043 wide, sinistral, near the edge of the acetabulum; cirrus sac, 0.124–0.211 long by 0.018–0.045 at the point of greatest width, transverse, between the crura and acetabulum, almost as long as body width; seminal vesicle, occupying the proximal ⅔ to ¾ of the cirrus sac, tapering to the region of the prostate, which is relatively poorly developed; ductus ejaculatorius, narrow, curving postero-medially, ending in the male papilla; male papilla, 0.005–0.019 long by 0.006–0.010 diameter, on the dorso-lateral wall of the genital atrium; ovary, 0.049–0.085 in largest diameter, subspherical to ovoid, sometimes lobed, dextral, largest diameter transverse or oblique; oviduct, passing posteriorly to the oötype which is slightly dextral and sometimes partly hidden by the acetabulum; testes, lateral, slightly posterior to the acetabulum, largest diameter longitudinal or oblique, 0.051–0.093; vasa efferentia, arising on the medial sides of testes, uniting just posterior to the proximal end of the cirrus sac; seminal receptacle, 0.011–0.034 in largest diameter, ovoid to spherical, postero-lateral to acetabulum, seen only in living specimens; eggs (living), operculate, unembryonated when shed, light yellow to brown, 0.019–0.025 by 0.011–0.014.

Variation in the relationships of structures in the region of the oötype is extremely great, due to the expansion of both the excretory vesicle and uterus. Eggs may become so numerous and organs so closely appressed that details of the excretory system and reproductive ducts are completely obscured. The disposition of the vitellaria is also subject to considerable variation. Typically, they consist of a narrow transverse band of small follicles which extends laterally on either side to the body wall, then turns posteriorly to form a ring around the posterior portion of the body (Text-fig. 1A). This ring may appear complete, but usually the two parts



TEXT-FIG. 1. Morphological variation in *Maritrema obstipum.*

are distinct at the posterior end as well as anteriorly. Variations from this pattern consist of anterior extension to the level of the oral sucker, forming a partial anterior ring instead of a posterior one (Text-fig. 1C); restriction of the fields to lateral masses only, at about the level of the acetabulum (Text-fig. 1B); and intergrade conditions between these two extremes (Text-fig. 1D). The two vitelline ducts meet near the midline, forming a somewhat enlarged vitelline reservoir ven-

trally. The uterus usually consists of the typical microphallid pattern of loops in the posterior part of the body, but in more gravid specimens extends anteriorly to the level of the oral sucker. When the excretory bladder is of maximum size, the uterus is restricted to the more anterior portions and contains darker brown ova on the anterior sinistral side. The distal end of the uterus enters the left side of the genital atrium near the male papilla. The excretory system consists of a very large, thin-walled, bicornuate excretory vesicle which may be distended to almost fill the posterior half of the body, and a system of branching, coiled tubules and flame cells whose formula is $2[(2+2)+(2+2)]$. The nature of the seminal receptacle has not been definitely established. It may be only an enlargement of the oviduct, and hence more accurately termed a fertilization chamber. Its form, size, and position have been observed only in living specimens, where the vigorously moving sperms make observation possible. Laurer's canal was not seen in either living or fixed (total and sectioned mount) specimens, probably due to crowded conditions near the oötype.

THE METACERCARIA (Plate I, Fig. 6)

The encysted, fully developed metacercaria may be free in, or attached by a few strands of connective tissue to the wall of, the isopod's hemocoel. They do not localize in any particular region, being found in all segments back of the head, and in the telson. The wall of the spherical cyst is extremely tough and inflexible, composed of a thin inner membrane, a heavy, radially striated middle layer, and a thinner fibrous outer layer, probably of host origin. Excystation experiments using gastric and pancreatic fluids never succeeded in breaking down the middle layer of the cyst wall, though the outer sheath was readily digested. The inner cyst membrane forms within a day or two of the time of cercarial penetration, but the thick middle layer is not formed until after about three weeks, when the worm has attained nearly 2/3 of maximum size. Cysts more than six weeks old have a fairly constant diameter of 0.208–0.257 mm., though cysts formed in the telson are always somewhat smaller in size, about 0.150 mm. Restricted space probably accounts for the dwarfing effect.

The morphology of the excysted metacercaria is almost the same as that of the adult, differing in the following characteristics: the excretory vesicle is relatively smaller; the uterus never extends anterior to the transverse band of the vitellaria; the oötype, ovary, and testes are somewhat more posterior in position. The degree of distention of the excretory vesicle, and the filling of the uterus with eggs are undoubtedly controlling factors in the position of the other organs. Variations of the vitellarian fields, as mentioned above, also have drastic effects on the location of the reproductive organs, particularly the cirrus sac which may come to lie in a more longitudinal position and extend posteriorly to the level of the anterior edge of the dextral testis (Text-fig. 1C). The excretory pattern is the same as that of the adult, $2[(2+2)+(2+2)]$.

THE CERCARIA (Plate I, Fig. 2)

Diagnosis. Small, opaque, distome, xiphidiocercariae covered densely with minute, flattened spines arranged in quincunx pattern. The following dimensions were determined in living cercariae, stained with neutral red or brilliant cresyl blue: body, 0.070–0.098 long by 0.036–0.056 wide, ovoid to elongate; tail, 0.064–0.098 long by 0.007–0.015 wide at the base,

leptocercous, lacking spines, finely annulated; oral sucker 0.021–0.027 long by 0.024–0.030 wide; stylet, 0.012–0.015 long by 0.003–0.004 wide, subcylindrical with lateral protuberances and a short ventrally directed point; pharynx, 0.009–0.015 long by 0.007–0.013 wide, spheroidal to trapeziform in shape, well developed, just posterior to the oral sucker; acetabulum, 0.013–0.024 in largest diameter, well developed, slightly smaller than oral sucker, bearing spines near the anterior margin; two large pairs of penetration glands, 0.018–0.030 in diameter, served by two large ducts opening at the anterior part of the oral sucker; two small pairs of penetration glands, 0.007–0.015 in diameter, emptying through two very slender (not always visible) ducts which open back of the two large duct openings.

The large gland cells lie at about the acetabular level with the smaller cells behind them. The esophagus is short and slender, communicating in front of the acetabulum with two short ceca which are not visible in all specimens. The cystogenous glands are well developed, generally distributed over the body, and contain a granular material which stains densely with neutral red. The excretory system consists of a fairly small, bicornuate vesicle opening ventrally through a short excretory duct at the posterior extremity of the body, and a system of slender, branching tubules and flame cells whose formula is $2[(1+1)+(1+1)]$. A mass of densely staining cells, just posterior to the acetabulum, is probably the genital primordium. The cercariae develop in ovoid to cylindrical sporocysts.

THE SPOROCYST (Plate I, Fig. 1)

Only natural infections of *Amnicola pilsbryi* were used for study. The parasite was identified by microscopic examination of the cercariae emerging or contained in the sporocysts. The sporocysts described here are probably the secondary generations. They are yellowish in color, saccate, usually elongate, and vary in size from 0.108–0.245 long by 0.074–0.105 wide. Their form is typically elongate, but smaller individuals may be only slightly ovoid. The walls vary considerably in thickness, becoming thinner in older specimens. The number of cercariae contained by an individual sporocyst may be as many as 24, but is usually 12 to 15. The more elongate sporocysts may show prominent constrictions which occasionally separate the posterior region almost completely. The birth pore is subterminal, and in large sporocysts, the region anterior to the pore may form a thick knob or linguaform projection. Motility is confined to an occasional wave of contraction passing over the body wall. The contained cercariae are active, and even show swimming movements of their tails. Emergence of the cercariae from the sporocyst is apparently haphazard, the larvae having been observed to emerge tail or oral sucker first without being pressed under a coverglass. The birthpore has been seen only in specimens in which emergence occurred, and its presence in earlier stages of development is doubtful.

The sporocysts occur free in the tissue of the digestive gland and gonads, and in such number that the upper whorls of the spire may be packed with them. As many as 188 sporocysts have been taken from a single snail, and more were probably present. In one snail only six sporocysts were found near the crystalline style.

THE MIRACIDIUM (Plate I, Fig. 4)

Upon expulsion, the ova of *Maritrema obstipum* usually have undergone not more than one cell division. Subsequent development at room temperature, using the method described above, is very slow. The size of the egg increases gradually

for about twenty days when a maximum of 0.024–0.030 by 0.012–0.015 is reached. The ovoid miracidium is fully formed at about 35 days and is sparsely covered with short cilia. Some evidence of epithelial plates has been seen, but their precise number and arrangement is not clear, due to increased opacity of the shell and the small size of the larva. Its anterior end is somewhat tapered, directed toward the operculum, and contains an opaque, loose mass of granules. Two less opaque bodies of more definite outline are found slightly posterior and lateral to the above granular mass. These bodies are identified as secretory or penetration glands, and contain a finely granular substance. Posterior and medial to the latter structures a dense elongated structure is identified as the "primitive gut". A single flame cell was seen postero-lateral to the latter structure in two cases. Granules probably of excretory origin are found in the space between the larva and the shell.

No experimental or spontaneous hatching of these eggs was observed, suggesting that ingestion is necessary for infection of the snail host. All attempts to infect wild, young snails have been unsuccessful.

V. *Levinseniella amnicolae* n. sp.

(Plate II)

A. OBSERVATIONS AND EXPERIMENTS

The cercariae of *Levinseniella amnicolae* are shed by the isolated gastropod host at all times of the day at a rate of 1 to 15 per day. Emergence may cease for one or two days and resume under apparently constant conditions. Behavior of these cercariae is very similar to that described for *Maritrema obstipum*. Instead of moving by alternate attachment of the two suckers, *L. amnicolae*, lacking an acetabulum, is restricted to the use of its tail in locomotion. Responses to light and movement, and length of life are essentially the same as observed in *M. obstipum*.

The incidence of molluscan infection is slightly greater for *L. amnicolae* than for *M. obstipum* in the autumn months, but shows little difference at other times of the year. Correlation of the time at which the first shedding snails were found, with the time at which the first mature infections appeared in the second intermediate host, *Asellus*, indicates a significant difference in the rate of metacercarial development of these two species. Though the encysted metacercariae of *L. amnicolae* are considerably larger, they attain maximum size only two to three weeks later than those of *M. obstipum*.

The second intermediate host, *Asellus communis*, was infected experimentally by the same method described above for *M. obstipum*. All hosts used were raised in the laboratory until after the experimentally produced metacercariae were found to be identical with those from natural infections. Subsequently, both experimentally and naturally infected isopods were employed. The metacercarial cysts grew rapidly in the experimentally infected isopods, attaining full size in 34 to 40 days. These cysts were found in all body segments and telson of the isopod. They were embedded in the connective tissue around the dorsal blood vessel, but in more mature infections, the cysts were usually free in the hemocoel. In one case, a single metacercaria of this worm was found encysted in the visceral mass of the snail host. The snail was otherwise uninfected indicating that the cercaria responsible was from another snail. The cyst was somewhat smaller than average

size; the worm excysted after 5 days in frog Ringer's solution and lived for 2 days thereafter under refrigeration.

Initial experiments involving possible homoiothermic vertebrate hosts were all negative. Consequently, attempts were made to infect a variety of fish, amphibians, and reptiles with equally negative results. A few cases of excystation occurred in salamanders and turtles, but no egg production was noted. One instance of naturally occurring excysted metacercariae was found in an adult *Triturus viridescens*. The condition of these worms was the same as the experimental forms.

In the first experiments with chicks, birds 7 days or older were used and dead encysted metacercariae were often found in the gizzard and upper intestine 1 to 3 hours after administration. The first positive avian infection was obtained in a young mallard duckling. Six gravid specimens were found in the ceca 10 days after 50 cysts were administered. Subsequently, positive infections were obtained with little difficulty in white mice and day-old chicks. *Levinseniella amnicolae* was found to localize in the ceca of birds and white mice, none occurring in the small intestine. In young chicks, the worms were sometimes found in smaller numbers in the yolk sac. Over 250 adult worms were recovered from the cecum of a white mouse 2 days after administration of over 300 cysts. In other mice, infected at the same time with approximately the same number of cysts, autopsy on the third, fourth, fifth, sixth, and seventh days, showed a progressive decrease until the seventh day, when no worms remained. The worms contained considerable numbers of eggs 20 hours after being eaten. Following ingestion of 200 to 300 cysts, infections persisted in chicks as long as 24 days, while in ducklings, worms were recovered after 38 days, the number of worms in each case being less than 10. Domestic ducks have been found naturally infected with considerable numbers of *L. amnicolae*, and it is probable that migratory ducks and many small mammals are susceptible to infection.

B. DESCRIPTION OF STAGES (ALL MEASUREMENTS IN MILLIMETERS)

THE ADULT (Plate II, Figs. 3 and 6)

Diagnosis. With the characters of the genus, *Levinseniella*. Fairly small ovoid to claviform distomes, with the posterior part of the body concave ventrally; cuticle with flattened spines in close-set quincunx pattern anteriorly, less dense in the middle third, and scattered to absent in the posterior third of the body; anterior end highly motile, the enlarged posterior portion, containing the reproductive organs, capable of little movement. Dimensions of fixed specimens as follows: body, 0.580–1.060 long by 0.180–0.395 wide; oral sucker, 0.062–0.098 long by 0.061–0.118 wide, subterminal, wider than long; acetabulum, 0.053–0.110, subcircular; prepharynx, 0.005–0.052 long, relatively short and slender; pharynx, 0.021–0.035 long by 0.018–0.030 wide, small, ovoid, longer than wide; esophagus, 0.130–0.228 long, slender; crura, 0.132–0.185 long, fairly broad; testes, 0.062–0.158 largest diameter, spheroidal to ovoid, at or slightly posterior to the level of the acetabulum; vasa efferentia, arising on the medial faces of the testes, passing anteriorly on the dorsal side where they join to form a short vas deferens; seminal vesicle, 0.015–0.047, antero-dextral to the acetabulum, diameter varying, with the degree of distention; ductus ejaculatorious, narrow, passing through a well developed prostate gland to the male copulatory organ; genital atrium, 0.050–0.080 in transverse diameter, complicated by four large, muscular pockets, lacking associated cuticularized structures; male copulatory organ, 0.024–0.043 long, arising from the antero-dorsal wall of the atrium, enlarged and flattened distally, weakly muscular; ovary, 0.056–0.146 in largest diameter, dextral, usually extending slightly anterior to the acetabulum; seminal receptacle, 0.021–0.028 long by 0.012–0.020 wide, ovoid, dextral to the oötype; Laurer's canal, arising from the oötype between the oviduct and seminal receptacle, passing postero-medially to its pore on the dorsal surface; eggs (living), 0.018–0.026 long by 0.009–0.012 wide, operculate, unembryonated.

The uterus exhibits coiling in the posterior portion of the body characteristic of the family, and enters the genital atrium on its posterior side; there is no differentiated metraterm. The uterus is invariably confined to the bulbous posterior portion of the body, never extending anterior to the ends of the crura. Observations of living specimens show the seminal receptacle to be a chamber separate from the oviduct and through which oöcytes do not pass. The vitellaria, composed of five to eight large follicles (0.030–0.085 in diameter) on each side of the body, are usually posterior to the testes. The primary vitelline ducts pass anteriorly, joining in the mid-ventral line, just back of the acetabulum, to form a common duct or reservoir. The excretory bladder is relatively small and bicornuate, the pattern of ducts is of the Mesostoma type, and the flame cell pattern is $2[(2+2)+(2+2)]$.

Type and paratype specimens of *Levinseniella amnicolae* have been deposited in the U. S. National Museum and bear the number U.S.N.M. Helminthological Collection, 48,766.

THE METACERCARIA (Plate II, Fig. 3)

The encysted metacercariae of *L. amnicolae* are found in the hemocoel of the isopod, *Asellus,* either attached by a few strands of connective tissue or free in the cavity. In cases of mixed infection, their larger size and chalk-white excretory granules easily distinguish them from cysts of *M. obstipum* which are smaller and contain more yellowish granules. The mature cysts are spheroidal to slightly ovoid and 0.358–0.463 in diameter, but smaller ones, 0.238–0.275 in diameter, occasionally are found in the telson.

The cyst wall is very refractile and inflexible. The inner membrane is delicate, less than 0.003 thick; the middle layer is thick, radially striated, inflexible, and resistant to digestive enzymes; the outer covering is relatively thick, closely applied to the middle layer, but is readily attacked by peptic and pancreatic solutions. Except for the absence of eggs in the uterus, the metacercariae are morphologically identical with the adult, and the dimensions given above may be applied to them.

THE CERCARIA (Plate II, Fig. 1)

Diagnosis. Small, "monostome," xiphidiocercariae, of the Ubiquita type. Dimensions of living cercariae, stained with neutral red, as follows: body, 0.070–0.138 long by 0.028–0.064 wide, ovoid to somewhat elongate; tail, 0.056–0.084 long by 0.007–0.012 wide at the base, leptocercous, lacking spines or setae; oral sucker, 0.018–0.034 long by 0.021–0.037 wide, subterminal; stylet, 0.012–0.015 long by 0.003–0.004 wide, simple, tapering to a sharp, ventrally directed point; penetration glands, 2 pairs on each side of the body, the posterior pair, 0.005–0.008 in diameter, the anterior pair, 0.010–0.016 in diameter.

The larger pair of penetration glands have prominent ducts which end in the oral sucker near the tip of the stylet, while the posterior pair have very delicate ducts opening lateral to the stylet. Cystogenous glands are not prominent even when stained with neutral red. The digestive system is not developed, nor is the acetabulum whose prominent primordium is found just posterior to the middle of the body, in close association with that of the reproductive system. The excretory system is of the Mesostoma type, and its formula is $2[(1+1)+(1+1)]$. The excretory vesicle is small, posterior in position, and empties through a short duct below the attachment of the tail.

THE SPOROCYST (Plate II, Figs. 2 and 4)

The sporocysts of *L. amnicolae* are ovoid to elongate, saccate and 0.124–0.288 long by 0.060–0.085 diameter. They may show deep constrictions, and in frog Ringer's solution the posterior end may separate spontaneously. All material studied was obtained from natural infections of *Amnicola pilsbryi,* and identified by means of the cercariae. The birth pore is subterminal, near the end bearing a bulbous projection, but is discernible only during the exit of a cercaria. The sporocysts vary in number, from a few in the digestive gland, to a condition in which the entire gonad and digestive gland are permeated and nearly obliterated by the infection. The number of cercariae contained by a single sporocyst varies from 0 to 14, more, usually being found in large specimens.

Attempts to culture the eggs of *L. amnicolae* were not successful, although development to the 10-cell stage was observed. After passage, the eggs slowly enlarge to a maximum size of 0.021–0.026 long by 0.011–0.013 wide at about the 20th day.

Levinseniella amnicolae is most closely related to the European species *L. pellucida* and *L. propinqua* described by Jägerskiöld (1908), but *L. amnicolae* is distinguished from the former by its relatively smaller digestive tract, larger ovary, smaller male copulatory organ, smaller eggs, and its occurrence in a different crustacean host than that reported for *L. pellucida* by Buttner (1951). *L. amnicolae* differs from *L. propinqua* in its longer intestinal crura, much larger pharynx, smaller acetabulum, smaller male copulatory organ, and lack of hooks and ribs found on the projections in the genital atrium of *L. propinqua.*

VI. Discussion

Since the present species of *Maritrema* was originally described from the intestine of the rock bass as *Microphallus,* and subsequently shifted to *Maritrema, Maritreminoides* and *Pseudospelotrema,* its taxonomic position was difficult to ascertain. Rankin (1939b) erected the genus *Maritreminoides* to contain the following species; *Maritrema nettae* (type), *M. medium,* and *M. obstipum.* The new genus was distinguished from *Maritrema* by its non-ring formed vitellaria, its long, protrusible cirrus, disparity of sucker size, and the more anterior extent of the uterine coils. The present study indicates that, with the exception of the nature of the cirrus, these characters are too variable to be used as generic distinctions.

Yamaguti (1939) erected the genus *Pseudospelotrema* to contain 3 new species, *P. japonicum, P. uriae,* and *P. cincli.* It was characterized by small oral and ventral suckers; a cirrus projecting into the genital atrium, which was described as a papilla in the type species, *P. japonicum;* non-ring formed vitellaria; and the uterus confined to the post-testicular area. This genus is very similar to *Maritreminoides,* but not identical with it since they differ in the anterior extent of the uterus, and in the character of the male copulatory organ. Disregarding this difference, Baer (1943) reduced *Maritreminoides* to synonymy with *Pseudospelotrema,* and Cable and Kuns (1951) concurred in this action. Hunter and Vernberg (1953) transferred *Maritreminoides nettae, M. obstipum,* and *M. medium* to *Pseudospelotrema.*

The disposition of the vitellaria in specimens of *M. obstipum* shows all the intermediate conditions between the lateral grouping typical of *Pseudospelotrema* and the ring-form typical of *Maritrema.* Another condition seen more rarely in present

specimens also demonstrates the extreme flexibility of the vitellarian pattern, the longitudinal bands extending anteriorly to form an almost complete ring in the forward half of the body (Text-fig. 1C). In *M. obstipum,* the uterus also varies in extent, from restriction behind the cirrus sac to complete filling of the anterior part of the body around the oral sucker. The extreme variability of these features in *M. obstipum* casts doubt upon the validity of their use as generic characters.

The present study indicates that distention of the excretory bladder exerts a strong influence on the location of other organs of the body. In specimens of *M. obstipum* freshly removed from the host gut, the excretory bladder is so voluminous that it almost fills the posterior half of the body. Shortly after removal to physiological saline, it usually becomes much smaller, rarely extending beyond the posterior edges of the testes. Concomitantly, the uterus, which is confined initially to portions of the body anterior to the excretory vesicle, becomes more posterior. The positions of the ovary, testes, and cirrus sac are also affected by the condition of the excretory bladder.

In the family MICROPHALLIDAE, Cable and Kuns (1951) have pointed out the evolutionary tendency toward reduction of the cirrus sac and eversible cirrus, the latter structure being reduced or replaced by a genital papilla. The family is also noted for remarkable uniformity of morphological pattern. Accordingly, the nature of the genital apparatus is of considerable taxonomic importance. Unfortunately, it is sometimes difficult to determine the nature of these small structures; consequently vague or inaccurate descriptions exist. In *Pseudospelotrema japonicum,* Yamaguti (1939) described the cirrus as a papilla, but his figure does not show this structure. This omission suggests that the structure actually was a small papilla, since an eversible cirrus is a fairly conspicuous organ and would probably have been figured if present. The genus *Maritrema,* characterized by the presence of a genital papilla rather than a cirrus, thus includes the concept of *Pseudospelotrema* Yamaguti, 1939. *Pseudospelotrema* is therefore reduced to synonomy with *Maritrema,* and the species *P. japonicum, P. uriae,* and *P. cincli* are transferred to *Maritrema.* In contrast to the three species described by Yamaguti, *P. ammospizae* Hunter and Vernberg, 1953, has a long, coiled, eversible cirrus and hence cannot be transferred to *Maritrema.*

As pointed out above, the genus *Maritreminoides,* Rankin, 1939, differs from *Pseudospelotrema* in respect to the nature of the cirrus. For this reason, Rankin's genus, characterized by a long protrusible cirrus, is retained to include *Pseudospelotrema nettae* (Gower, 1938) as type, and *Pseudospelotrema ammospizae* as *M. ammospizae* (Hunter and Vernberg, 1953).

Maritrema obstipum and *M. medium* were transferred to *Maritreminoides* by Rankin (1939b), and Baer (1943) reduced *Maritreminoides* to synonymy with *Pseudospelotrema. Pseudospelotrema obstipum* and *P. medium* are returned to *Maritrema* since both possess genital papillae. The position of *P. medium* is somewhat provisional because, although it is otherwise very closely related to *P. obstipum,* the nature of the copulatory organ has not been clearly demonstrated by either Van Cleave and Mueller (1932, 1934) or Sheldon (1938).

Hunter and Vernberg (1953) transferred *Maritreminoides medium* (Van Cleave and Mueller, 1932) and *M. obstipum* (Van Cleave and Mueller, 1932) to *Pseudospelotrema,* and left the question of their possible synonymy unsettled. The

present study shows that *P. obstipum* differs from *P. medium,* as described by Van Cleave and Mueller, (1932, 1934) and by Sheldon (1938) on the following morphological grounds: smaller cyst size; much shorter esophagus; much larger excretory vesicle; and a generally more anterior distribution of reproductive organs. Experimentally, *P. obstipum* was found to be capable of infecting a much wider range of vertebrate hosts than reported by Sheldon (1938) for *P. medium.* More convincing perhaps is the occurrence of metacercariae of the present species in the isopod, *Asellus,* while metacercariae of *P. medium* were reported from gills of the decapod, *Cambarus.* Crayfish from the locality of the present work were not infected and attempts to infect them in the laboratory were not successful.

In summary, the following changes are made in the taxonomy of *Maritrema, Maritreminoides* and *Pseudospelotrema*: 1) *Maritreminoides* is recognized as a valid genus characterized by the possession of an eversible cirrus and includes *P. nettae,* (type) and *P. ammospizae;* 2) *Pseudospelotrema* is suppressed as a synonym of *Maritrema* on the basis of Yamaguti's statement that the cirrus is a papilla in the type species, *P. japonicum;* 3) *Pseudospelotrema obstipum* and *P. medium* are distinguished from each other and returned to the genus *Maritrema* on the ground that they possess genital papillae.

The cercariae of *Maritrema obstipum* is of considerable interest, since it possesses an acetabulum, and other cercariae in the family Microphallidae are of the typical Ubiquita type in which an acetabulum is lacking. Morphological features similar to those of other microphallid cercariae are; four pairs of cephalic glands served by two types of ducts, a laterally asymmetrical stylet with point directed ventrally, and excretory system of the Mesostoma type and formula of $2[(1+1)+(1+1)]$. The cercaria of *M. rhodanicum,* which also possesses an acetabulum, was described by Carrère (1936), who referred it to the Armata group. He gave no figure of the larva, but his description is clear enough to convince the writer that the cercaria of *M. rhodanicum* is very similar to the one described here for *M. obstipum.* Assignment of the cercaria to the Armata type, with the connotations which the name implies, was perhaps an unfortunate choice of words, since the Armata cercariae differ in fundamental morphology from microphallid larvae. The cercaria of *M. caridinae* as described by Shibue (1951) is apparently a closely related form, possessing an acetabulum, but with only two pairs of penetration glands. This difference from other microphallid cercariae is probably due to a difference in rate of development of the ventral sucker. Its presence in these members of the genus *Maritrema* is a possible indication of a more primitive pattern of development, further indicated by their well developed digestive systems in the cercaria, and by the presence of the cirrus sac in the adult stage.

The long period of incubation necessary for development of the miracidium of *M. obstipum* agrees with the observations of Cable and Hunninen (1940) on *Spelotrema nicolli.* In contrast, Yamaguti (1934, 1935, 1939) reported embryonated eggs in specimens of various microphallid species which he described. Hunter and Vernberg (1953) described the eggs of *Pseudospelotrema ammospizae* as embryonated, and Shibue (1951) reported that the eggs of *Maritrema caridinae* were embryonated, but no description of the miracidium was given in either case. No detailed account of the miracidium in the family Microphallidae has been presented to date.

Yoshida (1916) described but did not name a small trematode which he found encysted in marine crabs, and Osborn (1919) named it *Microphallus japonicus.* Yoshida (1938) redescribed this species, showed the presence of a cirrus, an arcuate cirrus sac with complex, extrinsic muscle fibers, chitinous processes associated with the genital atrium, and stated that in his original description the dorsal aspect was mistaken for the ventral, with a corresponding reversal of symmetry. He erected the genus *Microphalloides* to contain *M. japonicus* as the type and only species, and it has remained monotypic to the present time.

The genus *Gynaecotyla* Yamaguti, 1939 (Syn. *Cornucopula* Rankin, 1939) as now accepted contains the five species, *G. jägerskiöldi, G. simillimus, G. adunca, G. squatoralae,* and *G. nassicola* (Syn. *C. sippiwissitensis*). It is distinguished by the possession of two ventral suckers; a two-horned copulatory organ, usually with cuticularized structures; an eversible cirrus; and the sinistral position of the ovary, and dextral position of the cirrus and genital atrium. Thus, *Gynaecotyla* is amphitypic in relation to all other genera of the family except *Microphalloides.* Hunter (1952) clearly described the cuticular plates associated with the genital atrium of *Gynaecotyla adunca* as well as an arcuate cirrus sac with peculiar extrinsic musculature. Yamaguti (1934) also indicated the presence of a complex of extrinsic muscle fibers associated with the cirrus sac of *G. squatoralae.* Yoshida's (1938) description of chitinous processes associated with the genital atrium of *Microphalloides* is not as precise as Hunter's, but it is clear that a similar apparatus is present in both genera. In essential and characteristic morphology, *Microphalloides* agrees closely with *Gynaecotyla,* and they are therefore regarded as congeneric. The difference in position of the vitellaria in *Microphalloides* is not considered a generic distinction, since it varies to a greater extent in a single species, *Maritrema obstipum.* The lack of a second acetabulum in *Microphalloides japonicum* can be explained in either of two ways. The second acetabulum may actually be lacking, as shown by Yoshida's description, possibly indicating an early stage in the evolution of the biacetabular genus. It may also have been very small, as in *Gynaecotyla simillimus,* and masked by the genital atrium with its associated cuticular apparatus. If the description of Yoshida (1938) is valid, the differences between *Microphalloides* and *Gynaecotyla* are insufficient to justify retention of both genera. Therefore, *Gynaecotyla* Yamaguti, 1939 is suppressed as a synonym of *Microphalloides* Yoshida, 1938 to include the six species, *Microphalloides japonicum* (type), *G. jägerskiöldi, G. simillimus, G. adunca, G. squatoralae,* and *G. nassicola.*[2]

Cable and Kuns (1951) presented a convincing discussion of the phylogenetic relationships of the family MICROPHALLIDAE. They showed that evolution of the copulatory structures has proceeded from primitive forms, possessing cirrus and cirrus sac, to forms in which these structures are lost and a male papilla and genital

[2] Hunter and Vernberg (1953, Trans. Amer. Micr. Soc. *72*: 163-170) reported on the life history of *Gynaecotyla adunca* (Linton, 1905). Their account is unique for a species in the family Microphallidae, since the described cercaria is of the Cercariaeum type, and almost identical with the larval zoogonid, *Cercariaeum lintoni,* as described by Shaw (1933, Biol. Bull. 64: 262–275). Hunter and Vernberg stated that the life history of *G. nassicola* (Cable and Hunninen, 1938) as described by Rankin (1940b), in which a ubiquitous cercaria is described, is probably in error. Furthermore they reduced *G. nassicola* to synonymy with *G. adunca.* Since the infection experiments of Rankin and also Hunter and Vernberg were not based on laboratory raised intermediate hosts, further and better controlled investigations will be required to resolve the present confusion.

atrium have been developed and secondarily elaborated. They suppressed the family MARITREMATIDAE Baer, 1943, on the ground that presence of a cirrus sac, and ring-formed pattern of vitellaria are not characters of familial distinction. This position is supported by the present report which shows the vitellaria of *Maritrema obstipum* to be extremely variable, and the life histories of members of the two subfamilies to be identical.

The writer concurs with the scheme of phylogeny presented by Cable and Kuns, except for changes in the position of genera whose taxonomy has been altered above. The position of *Maritreminoides* is probably more primitive than they have indicated, since they regarded it as a synonym of *Pseudospelotrema*. *Microphalloides* is substituted in the position of *Gynaecotyla* and is regarded as a primitive type, since it possesses both cirrus pouch and cirrus.

VII. SUMMARY AND CONCLUSIONS

The life histories of *Maritrema obstipum* and *Levinseniella amnicolae* are reported. *Levinseniella amnicolae* is described as a new species, and is differentiated from closely related forms. Larval and adult stages of both species are described from natural and experimental hosts. The cercariae of both species emerge from the snail, *Amnicola pilsbryi* Walker, 1906, penetrate and encyst in the aquatic isopod, *Asellus communis* Say, 1818, and experimentally reach sexual maturity in a variety of birds and mammals. Natural infections of both species were found in domestic ducks collected in the infected pond. The adult of *M. obstipum* is usually found in the small intestine, and *L. amnicolae* localizes chiefly in the cecum.

The action of Cable and Kuns (1951) in suppressing the family MARITREMATIDAE Baer, 1943 is supported by additional experimental evidence. The genus *Pseudopelotrema* Yamaguti, 1939 is suppressed as a synonym of *Maritrema* Nicoll, 1909, and the species *Pseudospelotrema japonicum, P. uriae,* and *P. cincli* are transferred to *Maritrema* as *Maritrema japonicum, M. uriae,* and *M. cincli.* The genus *Maritreminoides* Rankin, 1939 is restored to contain *Pseudospelotrema nettae* and *P. ammospizae* as *Maritreminoides nettae* (type) and *M. ammospizae. Pseudospelotrema obstipum* and *P. medium* are returned to the genus *Maritrema.*

Maritrema obstipum and *M. medium* are differentiated from each other on both morphological and bionomic grounds. The cercaria of *M. obstipum* is shown to differ slightly from the Ubiquita type described for other members of the family MICROPHALLIDAE, and is regarded as similar to the cercaria of *M. rhodanicum* described by Carrère (1936), and *M. caridinae* described by Shibue (1951). These three larvae possess an acetabulum and digestive tract, but otherwise are similar in fundamental morphology to cercariae of other microphallid species.

The genus *Gynaecotyla* Yamaguti, 1939 is suppressed as a synonym of *Microphalloides* Yoshida, 1938. Accordingly, *Gynaecotyla jägerskiöldi, G. simillimus, G. adunca, G. squatoralae,* and *G. nassicola* are transferred to *Microphalloides* as *Microphalloides jägerskiöldi, M. simillimum, M. aduncum, M. squatoralae,* and *M. nassicola.* The type of the genus is *M. japonicum.*

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Explanation of Plates

(All dimensions in millimeters)

Plate I. (All figures concern *Maritrema obstipum*)

Fig. 1. Stages in the development of sporocysts in *Amnicola pilsbryi;* drawn in outline, free-hand.

Fig. 2. Xiphidiocercaria after emergence from snail, ventral aspect; and lateral aspect of stylet; drawn free-hand.

Fig. 3. Cross section of adult through the genital atrium, showing the male papilla; drawn with the aid of the camera lucida.

Fig. 4. Unhatched miracidium, based on several camera lucida drawings of living specimens.

Fig. 5. Gravid adult in ventral aspect, showing the extent of the uterus and excretory vesicle in specimens freshly removed from the host. Drawn free-hand and based on observations of living and permanently mounted specimens.

Fig. 6. Slightly flattened, permanently mounted metacercaria, showing details of morphology in ventral view. Excretory ducts, flame cells and seminal receptacle taken from observations of living specimens. Major structures drawn with the aid of the camera lucida, and details drawn in free-hand.

Plate II. (All figures concern *Levinseniella amnicolae*)

Fig. 1. Ventral aspect of cercaria shed by snail, and lateral aspect of the stylet; drawn free-hand.

Fig. 2. Stages in the development of sporocysts, drawn in outline, free-hand.

Fig. 3. Cross section of adult through the genital atrium, showing the male copulatory organ; drawn with the aid of the camera lucida.

Fig. 4. The living egg, drawn with camera lucida.

Fig. 5. Slightly flattened, permanently mounted metacercaria, showing details of morphology from ventral aspect. Excretory pattern added from observations of living specimens. Drawn with the aid of the camera lucida.

Fig. 6. Permanently mounted adult, unflattened, showing the position of uterine coils; ventral view. Drawn with the aid of the camera lucida.

Lettering: Ac, acetabulum; Ce, digestive cecum; CS, cirrus sac; DE, ejaculatory duct; Es, esophagus; EV, excretory vesicle; FC, flame cell; GA, genital atrium; GM, germinal mass; GP, genital pore; LC, Laurer's canal; MC, male copulatory organ; MG, Mehlis' gland; MP, male papilla; Od, oviduct; OS, oral sucker; Ov, ovary; PA, primordium of acetabulum; PG, penetration or cephalic gland; Ph, pharynx; PP, prepharynx; Pr, prostate gland; SR, seminal receptacle; St, stylet; SV, seminal vesicle; Ts, testis; Ut, uterus; VD, primary vitelline duct; Vi, vitellaria; VR, vitelline reservoir.

PLATE I

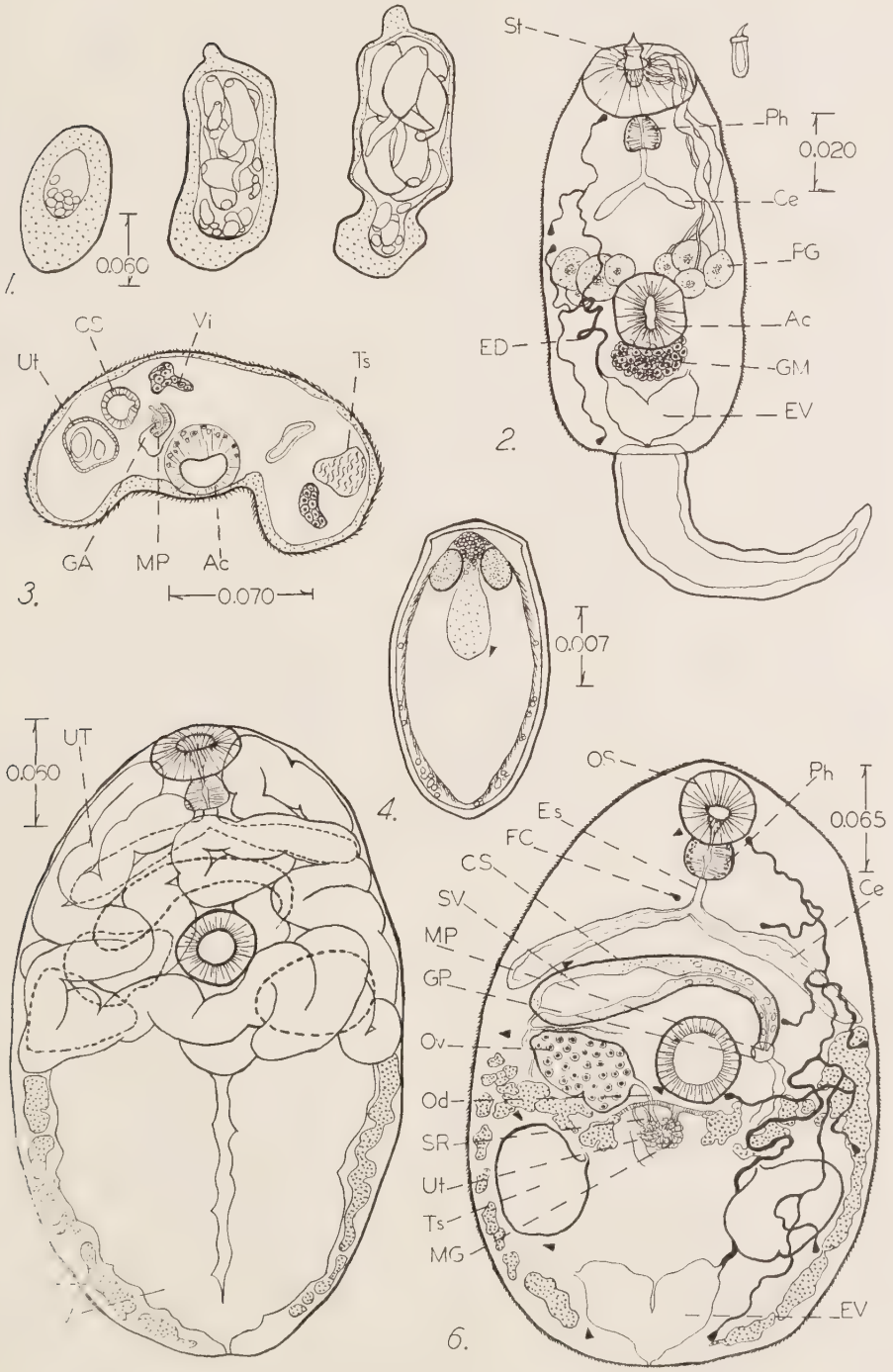
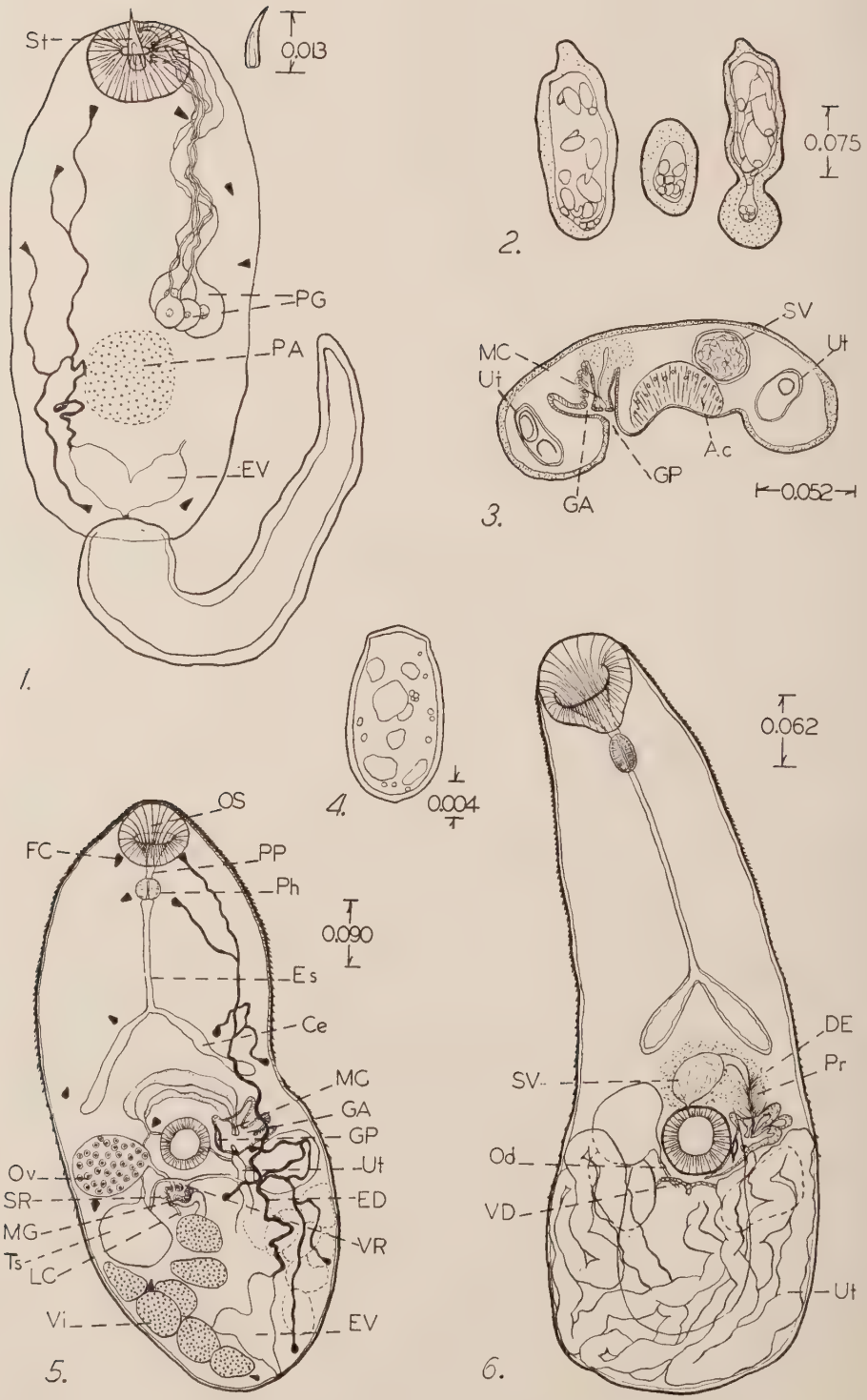

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PLATE II


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THE NONTRANSMISSION OF THE RELAPSING FEVER SPIROCHETE, *BORRELIA DUGESII* (MAZZOTTI, 1949) BY THE ARGASID TICK *ORNITHODOROS TURICATA* (DUGÈS, 1876)

GORDON E. DAVIS[1] AND LUIS MAZZOTTI[2]

In 1949, one of us (L.M.) reported the transmission of *Borrelia dugesii* by *Ornithodoros turicata.* As *Ornithodoros dugesi* is the normal tick vector of this spirochete, this was the first reported major break in the specific tick-spirochete relationship in the Western Hemisphere.

In 1952, the senior author of this report requested a strain of *B. dugesii* for further studies. As *O. dugesi* was not available the strain was forwarded in *Ornithodoros coriaceus,* from which spirochetes have never been recovered, as collected in nature, and which did not transmit the spirochetes by feeding. The *O. coriaceus* were, therefore, triturated in a minimal amount of physiologic saline and the triturate was injected into two white mice from which a strain of spirochetes was readily recovered.

To confirm the 1949 report and to determine whether or not *O. turicata* from the United States would also transmit *B. dugesii,* ten *O. turicata* (United States stock), ten *O. turicata* (Mexican stock), and ten *O. dugesi,* as a control on transmission, were fed on a mouse infected with the *O. coriaceus* triturate strain. The *O. turicata* were in the first nymphal stage and the *O. dugesi* in the second nymphal stage as ticks of this species do not feed in the first nymphal stage.

After molting, the ticks were tested for infectivity by feeding them individually on white mice. There was transmission by 6 of the 10 ticks in each of the *O. turicata* stocks. Following an additional molt, the ticks were tested in a similar manner with a resulting transmission by 8 of 10 ticks of the U. S. stock and transmission by all of the Mexican ticks. However, there was no transmission by *O. dugesi* following two successive test feedings.

It then seemed possible that the *O. turicata* used in the original experiment were carrying spirochetes of their own and that they had transmitted them instead of *B. dugesii* and that the *O. coriaceus* had been fed on a mouse infected with this strain. The above recorded results, with the tentative interpretation, were then communicated to the junior author.

As both authors of this report were equally desirous of confirming or disproving the original report, a special trip was made to the State of Coahuila in northern Mexico to obtain *O. dugesi* so that a fresh strain of spirochetes might be recovered. A true strain of *B. dugesii* was recovered in Mexico by feeding these ticks on white mice. *O. dugesi* from the new stock were then fed on a mouse infected with this strain and forwarded to the senior author. Spirochetes were readily recovered by feeding the ticks on white mice. Studies were then continued with the strain recovered from the *O. coriaceus* triturate, and experiments comparable to the original experiment were initiated with the new strain of *B. dugesii.*

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FURTHER STUDIES WITH THE SPIROCHETE RECOVERED FROM THE *O. coriaceus* TRITURATE

1. Quantitative transovarial transmission: At the third test feeding of the Mexican *O. turicata,* which also resulted in 100 per cent transmission, there were five females and five males. As up to 100 per cent transovarial transmission of spirochetes has been shown in *O. turicata* (Davis, 1943), each of the five females was allowed to mate with one of the five males and the females were held for oviposition.

Nine hundred sixty larvae, the progeny of these females (♀2, 210 larvae; ♀3, 210; ♀7, 143; ♀8, 293; ♀9, 104) were tested for infectivity in 190 groups of five each and in three groups of four, three, and three, respectively, by feeding on white mice. Spirochetes were demonstrated in the tail-blood of each of the 193 mice on the fourth day after tick feeding.

2. Immunity tests: Fifteen mice were infected by feeding *O. turicata* which were carrying the *O. coriaceus* triturate strain and five were infected by feeding ticks which were carrying a Mexican *O. turicata* strain from the same area as the *O. turicata* used in the original experiment. (This strain had been received from Mexico five years previously and had been carried in the meantime as a part of the spirochete collection in its natural vector, *O. turicata.*) Thirty days after spirochetes had appeared in the peripheral blood, the mice were challenged subcutaneously with 0.5 ml. of citrated blood containing the Mexican *O. turicata* spirochete. The tail-blood of each mouse was examined daily until a relapse had occurred in two control mice which had been injected with the same inoculum. All test mice were immune as spirochetes were not found over this period.

Twelve mice were infected by feeding *O. turicata* which were carrying the *O. coriaceus* triturate strain and thirty days later similarly challenged with *Borrelia dugesii.* There was no immunity as spirochetes appeared in the tail-blood of each mouse on the third day at which time spirochetes were first demonstrated in the blood of two control mice.

STUDIES WITH THE FRESHLY RECOVERED *B. dugesii* STRAIN

To repeat the original (1949) experiment, ten *O. turicata* (U.S.A. stock) and ten *O. dugesi* were fed on a mouse infected with this strain. Following the first and second testing of *O. dugesi,* after successive molts, there was transmission by 1 and by 9, respectively, while there was no transmission by *O. turicata* over three test feedings.

In a second experiment initiated five days after the above, ten *O. turicata* and ten *O. dugesi* fed on another mouse infected with the strain recovered by feeding *O. dugesi.* The ticks were tested for infectivity in the same manner as in experiment I. Following the first, second, and third test feedings of *O. dugesi,* there was transmission by 5 ticks, while following three test feedings of *O. turicata,* again there was no transmission.

In the combined results of the two experiments with the *O. dugesi* strain there was, therefore, transmission by 14 out of 20 *O. dugesi* and none by *O. turicata.*

The courses of infection by the *O. coriaceus* triturate strain, a known *O. turicata* strain from Mexico, and the strain recovered by feeding *O. dugesi* were followed in adult white mice and in adult and new-born (24-hour old) guinea pigs. The

O. coriaceus triturate strain and the Mexican *B. turicatae* strain appeared in the peripheral blood of mice as early as the fourth day following tick feeding while the *B. dugesii* strain appeared much later, most frequently on the eighth day. The guinea pigs reacted to the first two strains with the characteristic relapse phenomenon typical of *B. turicatae* from the United States (Davis, 1939) while neither adult nor new-born guinea pigs were susceptible to the strain recovered by feeding *O. dugesi.*

DISCUSSION

The maintenance of a non-infective (spirochete-free) stock of ticks is at times difficult. The initial interpretation, as mentioned above, was based in part on an experience previously reported (Davis, 1948); "Over a period of years in specificity studies, the reviewer has used a New Mexico stock of *O. turicata* none of which has ever been shown to be infective. In a recent experiment, early stage nymphs of this stock were not available, consequently specimens from another stock which had proved non-infective on two successive test feedings were used. At the first test feeding following a blood meal on a mouse infected with a spirochete foreign to *O. turicata,* spirochetes appeared promptly in the peripheral blood of the test mice. This cast suspicion on the "clean" stock being used. Thirty-five of the remaining ticks of this stock were, therefore, tested in seven groups of five each. All groups were shown to be carrying their own spirochetes."

As spirochetes foreign to the tick species may survive within the tick over varying periods of time it is an unreliable procedure to use a strain recovered from a triturate of the ticks rather than by tick feeding.

SUMMARY

A previous report that the relapsing fever spirochete, *Borrelia dugesii* was transmitted by *Ornithodoros turicata,* as well as by its normal tick-vector *Ornithodoros dugesi,* constituted the first major break in the specific tick-spirochete relationship in the Western Hemisphere. As *O. dugesi* were not available the spirochete thus transmitted was forwarded to the senior author of this report in *Ornithodoros coriaceus,* for further studies. The ticks were triturated in a minimal amount of physiologic saline and the triturate was injected into two white mice from which a strain was readily recovered. This strain was then studied by: (1) Transmission experiments with *O. turicata* and *O. dugesi;* (2) quantitative transovarial transmission in *O. turicata;* (3) reciprocal cross-immunity tests; and by (4) comparative studies with a known Mexican *O. turicata* strain and a true *B. dugesii,* recovered by feeding *O. dugesi.* By these methods it was shown that the spirochete was not *B. dugesii* but *B. turicatae.* When a true *B. dugesii* was tested for transmission by *O. turicata* and *O. dugesi* there was no transmission by *O. turicata.* It was concluded that the *O. turicata* used in the original experiment were carrying spirochetes of their own and that they had transmitted these instead of *B. dugesii.* The difficulty of maintaining a spirochete-free stock of ticks is discussed and the unreliability of using a strain of spirochetes obtained from a triturate of ticks instead of by feeding the ticks is stressed.

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RESEARCH NOTES

RETINELLA INDENTATA (SAY), A FIRST INTERMEDIATE HOST OF *ENTOSIPHONUS THOMPSONI* SINITSIN (1931) (TREMATODA: BRACHYLAEMIDAE)

The brachylaemid trematode, *Entosiphonus thompsoni,* is found in the small intestine of the short-tailed shrew, *Blarina brevicauda,* and, infrequently, in the white-footed mouse, *Peromyscus leucopus noveboracensis.*

Sinitsin (Ztschr. Parasitenk. 3 (4) : 821–822: 1931e) briefly described the metacercaria recovered from *Gastrodonta suppressa* (= *Ventridens suppressus*).

In an attempt to find the natural first intermediate snail host, a survey was made of shrews and terrestrial snails in the vicinity of Ann Arbor, Michigan, in an area known to have snails which harbored the metacercariae of this brachylaemid. Of 85 shrews examined during the period between Mar. 1952 and Jan. 1953, 44 showed both mature and immature forms of the parasite. Mature specimens were also recovered from two out of 49 *Peromyscus* examined during the same period. The following table summarizes the snails collected and autopsied for intramolluscan stages of *E. thompsoni:*

Snail Hosts	No. examined	Intramolluscan infections			
		With metacercariae		With sporocysts	
		E. thompsoni	Other species	*E. thompsoni*	Other species
Anguispira alternata	14	0	1	0	0
Cionella lubrica	11	0	0	0	0
Deroceras laeve	68	1	3	0	0
Mesodon thyroidus	5	0	0	0	0
Retinella indentata	8	1	2	1 (?)	0
Stenotrema monodon	204	0	11	0	0
Triodopsis multilineata	113	0	2	0	0
Succinea ovalis	173	3	11	0	1
Zonitoides nitidus	329	35	18	0	4
Ventridens ligera	1185	128	646	0	19

Each of 19 *V. ligera* collected in nature, which were actively shedding brachylaemid cercariae, turned out to have a brachylaemid other than *E. thompsoni.* This was true for the natural sporocyst infections in *S. ovalis* and *Z. nitidus.* On July 9, 1952, one of eight *Retinellas* collected was found with a heavy infection of branching sporocysts. Cercarial stages, however, were very immature, and there was no opportunity to make a study of morphological features.

In view of these observations, eggs from adult flukes were fed to laboratory-reared specimens of *R. indentata, Z. nitidus, V. ligera,* and *S. ovalis.* Branching sporocysts with mature cercariae developed only in *R. indentata.* Under laboratory conditions, cercariae were shed 60–70 days after feeding of eggs. Metacercariae were obtained upon transfer of cercariae to suitable laboratory second intermediate snail hosts. Two sexually mature adults of *Entosiphonus* were recovered from the small intestine of a laboratory *Peromyscus* 21 days after 10 metacercariae, 85 days old, were fed to this host.

These experiments indicate that *R. indentata* may serve as an experimental host in the life history of *E. thompsoni.* Metacercariae were found in four land snails not previously recorded as second intermediate hosts of this brachylaemid.—John B. Villella, *Department of Zoology, University of Michigan, Ann Arbor, Michigan.*

CYSTICERCUS FASCIOLARIS IN THE SYRIAN HAMSTER

In the course of experimental investigations of the sarcoma producing power of *Cysticercus fasciolaris* in the liver of the white rat and while rechecking the life cycle of this parasite, viable ova from gravid proglottids of *Taenia taeniaformis,* developed in laboratory cats, were fed to thirty Syrian hamsters and sixty white rats. All of the rats later revealed infection with *Cysticercus fasciolaris* but not a single cysticercus was found in any of the hamsters. Moreover, fecal examinations revealed that the taenia eggs were passed through the intestinal tract of the hamsters with the embryophore intact.

These findings suggested that the hamster might be successfully infected with the larval form of *Taenia taeniaformis* if the digestive processes in the hamster were aided by some in

vitro pre-digestion of the embryophores. Accordingly, ova from gravid proglottids of this cestode were subjected at body temperature (98–99 degrees F.) to an artificial gastric juice (0.5 per cent HCl–0.7 grams pepsin). No visible effect on the embryophores was demonstrable after four hours of this treatment. Other eggs were then placed in a digestion medium consisting of equal parts of (1) 2.0 per cent aqueous solution of desiccated ox bile and (2) 1.0 per cent aqueous solution of pancreatin adjusted to a pH 7.2–7.6. Embryophores disintegrated in from five to twenty minutes in this medium, releasing active oncospheres. The newly released oncospheres were placed in physiological saline (0.85 per cent NaCl) and introduced into a series of hamsters as follows: five hamsters received oncospheres per os; five received oncospheres injected into the portal vein; five were given injections of oncospheres directly into the liver; and five received oncospheres into the body cavity. All animals survived these procedures and after two months, careful autopsy including sectioning of the liver, revealed that none of these animals carried *Cysticercus fasciolaris.*

Repeated experimental attempts to infect the hamster with viable *Taenia taeniaformis* ova have failed. One, as yet unexplained, infection with a single *Cysticercus fasciolaris* strobilocercus 1.4 cm. long was found in the liver at autopsy of a hamster from a separate series which had been experimentally infected with *Trichinella spiralis.* The rostellum of this strobilocercous was armed with a double row of forty hooklets. The large hooklets averaged 0.400 mm. in length while 0.260 mm. was the average length of the small hooklets. Among the possibilities to account for the infection are (1) the chance ingestion of an extremely viable egg or (2) some peculiar phenomena provided by the trichinae or provoked by the concomitant presence of the two parasites which was conducive to invasion of the liver by an active oncosphere released after accidental ingestion. Further experimentation is in progress in an attempt to infect the hamster with *Cysticercus fasciolaris.*—Wayne W. Wantland, *Illinois Wesleyan University, Bloomington, Illinois.*

A NOTE ON *GIARDIA DUODENALIS* VAR. *PEROGNATHI* FILICE AND *ENDAMOEBA* (=*ENTAMOEBA*) *DIPODOMYSI* HEGNER IN CALIFORNIA RODENTS

Fresh fecal preparations and fecal smears stained with iron-haematoxylin from heteromyid rodents of California have been examined. A *Giardia,* morphologically similar to *Giardia duodenalis* var. *perognathi* Filice, 1952 was found in material taken from the small intestine of *Dipodomys agilis agilis* Gambel and *D. merriami merriami Mearns.* Other than Filice (1952, Univ. Calif. Publ. Zool., 57: 53–143), the only previous record of *Giardia* in heteromyid rodents is that of Herman (1943, J. Parasit., 29: 423).

Hegner (1926, J. Parasit., 12: 203–205) found trophozoites of an amoeba in *Dipodomys spectabilis* ssp. from New Mexico which he named *Endamoeba* (=*Entamoeba*) *dipodomysi.* Trophozoites, agreeing in most respects with those of Hegner, were obtained from *Dipodomys panamintinus mohavensis* (Grinnell), *D. merriami merriami* Mearns, *D. heermanni morroensis* (Merriam) and *D. agilis agilis* Gambel. A few nuclei, however, possessed uneven layers of irregular spaced chromatin granules on the nuclear membranes and 5 or 6 distinct linin strands extending from the karyosome to the nuclear membrane. Oval cysts, 13.0 to 16.0 (av. 14.2) μ long by 10.8 to 12.2 (av., 11.2)μ wide were obtained from fecal pellets of the same kangaroo rats harboring trophozoites. All cysts contained from 6 to 8 nuclei and were similar to the trophozoite nucleus described by Hegner with the exception of size. The writer assumes that these are the previously undescribed cysts of *Endamoeba* (=*Entamoeba*) *dipodomysi* Hegner, 1926. The incidence of infection of this amoeba in the kangaroo rats was between 40 and 50 per cent. This appears to be remarkably high when the arid desert habitat of the hosts is taken into consideration.—David J. Doran, *Bureau of Animal Industry, Beltsville, Maryland.*

COMPARATIVE SUSCEPTIBILITY OF *ANOPHELES QUADRIMACULATUS* "STRAINS" TO DOMESTIC *PLASMODIUM VIVAX*

The historic differences in the incidence of malaria of the delta and rice-growing regions of Arkansas have created speculation regarding possible physiological variations in *Anopheles quadrimaculatus* from these two areas. According to Barbar, et al (Pub. Health Rep. 41: 2527–2549; 1926), Italian malariologists have suggested that a normally efficient malaria vector may become less susceptible to infection through altered conditions in rice fields. Barbar and co-workers attempted to determine by infection experiments whether *A. quadrimaculatus* from the rice field area of Stuttgart, Arkansas, was susceptible to malaria infection. A total of 60 females was dissected from 6 lots of rice field mosquitoes fed at different times on human malaria carriers with demonstrable gametocytes of *Plasmodium falciparum.* The percentage

infection of these 6 lots was 0, 58, 45, 64, 0, and 0. Thus these workers concluded that *A. quadrimaculatus* from rice fields was susceptible to human malaria parasites.

In the present instance, *A. quadrimaculatus* was collected from breeding areas in the Mississippi delta at Helena, Arkansas, and from rice fields at Stuttgart, Arkansas. These "strains" were compared with the colonized (Q-1) strain to determine susceptibility to St. Elizabeth *Plasmodium vivax*. Four comparative feedings were made on neurosyphilitic patients being given malaria therapy. The results of these infective feedings may be summarized as follows:

Mosquito "Strain"	Mosquitoes		Per cent infected
	Dissected	Infected	
Q-1	96	75	78
Delta	71	53	75
Rice field	62	48	77

These data suggest approximately equal susceptibility to domestic *P. vivax* in delta, rice field, and Q-1 "strains" of *A. quadrimaculatus*. These findings were likewise borne out by the comparative intensities of oöcysts upon the mosquito midguts. If this is the true situation, it substantiates the opinion of Barbar (l. c.) that the relative freedom from malaria formerly enjoyed in the Arkansas rice field region, at a time when high rates existed in the delta area, was probably due more to human than to mosquito factors.

The writer wishes to acknowledge the aid of Drs. Martin D. Young, Scientist Director, and Robert W. Burgess, Medical Entomologist, National Institutes of Health, Laboratory of Tropical Diseases, Columbia, South Carolina, in whose laboratory the infection experiments were accomplished.—Leo Kartman, *Communicable Disease Center, Public Health Service, Department of Health, Education, and Welfare, Atlanta, Georgia.*

NOTE ON THE OCCURRENCE OF THE TICK *IXODES AFFINIS* NEUMANN IN THE UNITED STATES

Among numerous lots of ticks collected from various Florida hosts by Joseph C. Moore and sent to the Rocky Mountain Laboratory for identification was a single female of *Ixodes affinis* Neumann 1899, a species which has not been previously reported from the United States. The specimen was found on a lynx, *Lynx rufus floridanus,* at Gulf Hammock, Levy County, July 17, 1948, Dave Swindell, collector. Further collecting in northern Florida by one of us (A. J. R.) has resulted in the finding in the same locality of two additional females, one on a dog, July 14, 1951, and the other on a lynx, October 28, 1951. The discovery of this tick in the United States is of interest in that the previous most northerly record of its occurrence was from southern Mexico. The Rocky Mountain Laboratory has two unpublished records from Ocozocoautla in the State of Chiapas; 1 female from the coati, *Nasua narica,* and 1 male and 7 females from the deer, *Mazama sartorii,* both in December 1946, Miguel Alvarez del Toro, collector. The few previous records of the species have been from Guatemala, Costa Rica, and Panama, and from Brazil, where it has been reported under the synonymic name *Ixodes ricinus aragãoi* Fonseca 1935. Other recorded hosts are deer, *Mazama simplicicornis* and *Odocoileus chiriquensis,* ocelot, *Felis pardalis,* and puma, *Felis* sp.

The finding of *I. affinis* in Florida in three separate instances, once in 1948 and twice in 1951 as recorded above, indicates that the species is actually established in that State.—Glen M. Kohls, *U. S. Public Health Service, National Institutes of Health, National Microbiological Institute, Rocky Mountain Laboratory (Hamilton, Montana)* and Andrew J. Rogers, *Department of Entomology, University of Florida, Gainesville, Florida.*

A KOREAN CASE OF INFESTATION WITH *THELAZIA CALLIPAEDA* WITH NOTES ON HUMAN THELAZIASIS

In December of 1945, Dr. Kim Hi Choon, ophthalmologist of the Women's Medical College Hospital, Seoul, extracted one adult male nematode from the left eye of an 11-months old Korean boy. The patient was born and had lived in Ch'aryongwan, Pyonganpukto province in the extreme northwestern part of Korea until only a few weeks before examination. The mother remarked that she had previously removed one worm and disposed of it and that a third worm still remained in the eye. Dr. Kim located the worm in the inner angle of the eye, and upon applying pressure, observed that it glided swiftly across the eyeball to the outer

angle of the eye. He thought the cream-colored worm had been lodged in the lachrymal sac until disturbed by the pressure. The eye was normal in every other respect, and the right eye likewise revealed no pathology. Dr. Kim noticed that the boy's eye did not blink when the worm crossed the eyeball. Some months later when the specimen was turned over to Dr. Ra San Yung, parasitologist at Seoul University Medical College, he identified it as an adult male *T. callipaeda*. This case appears to be the second report of human infestation with *T. callipaeda* from Korea.

T. callipaeda is a spirurid nematode occurring commonly under the nictitating membrane of dogs in the Orient, but cases of human infestation have occasionally been encountered in China, Korea and India. In man the worms are found usually in the conjunctival sac of the eye. Among the symptoms produced by their presence are foreign body sensation, aching, intense pain, and excessive lacrimation. It is believed that the cornea may gradually become opacified by intermittent passage of the worms across its surface. The life cycle of *T. callipaeda* is unknown, but since closely related spirurids are known to utilize arthropod vectors, a similar cycle is postulated for *T. callipaeda*. Krasten (1952, Dokladi Akademii Nauk SSSR, 82: 829) reported that *Thelazia gulosa* and *T. skrjabini*, both parasites in the eyes of cattle, utilize *Musca amica* as an intermediate host. Two instances of human infestation with one other species of *Thelazia, T. californiensis,* have been reported from California, U.S.A. (Kofoid et al, 1937, Univ. Calif. Pub. Zool., 41: 225; Hosford et al, 1942, Arch. Ophth., 27: 1165.)

Hsü (1933, Arch. F. Schiffs-u.-Trop.-Hyg., 37: 363) reviewed four cases of human infestation from China, three with adult worms and one with larvae. Liang (1945, Chinese M. J., 63: 70) reported a case of infestation of the eye with *T. callipaeda* from Kweiyang, Kweichow; six worms (four males and two females) were extracted from the left eye of a Chinese truck driver. Chang (1948, Nat. Med. J. China, 34: 45) reported another case from Peiping.

A search of literature on human thelaziasis in Korea disclosed a report of a case by Nakata (1934, Jour. Chosen Med. Assoc., 24: 939) in which a single female worm was extracted from the eye of a Korean girl in 1927, at Hwanghedo province, north-central Korea.

Friedmann (1948, Antiseptic, 45: 620; 1949, Ophthalmologica, 118: 193) made the first report of a case of human infestation with *T. callipaeda* from India, in a 15 months old native female.

Thus, the recorded distribution of *T. callipaeda* infestation in man, formerly limited to China, has been extended to Korea and India.—E. I. Sillman, *Department of Zoology, University of Michigan, Ann Arbor, Michigan.*

THE IDENTITY OF *RHIPICEPHALUS SANGUINEUS* IN THE U. S. A.

The brown dog tick *Rhipicephalus sanguineus* (Latr.) 1806 is considered to have a world-wide distribution and a large range of hosts. Recently we have found that *R. sanguineus* includes two distinct species. The name *R. sanguineus* s. str. was attached to the species which is most common on the dog, and the name *R. secundus* was given to the other (Feldman-Muhsam 1952, Bull. Res. Council of Israel **2**: 187–194).

The adult females of *R. sanguineus* (Latr.) s. str. and *R. secundus* Fel.-Muh. are readily distinguished by the form of the genital aperture in cleared and mounted preparations. The genital aperture of *R. sanguineus* s. str. has a large round, widely open cup, whereas in *R. secundus* the form of the cup is less round and the inner opening of the cup is much narrower. We are unable to distinguish between the males of the two species. The larvae and nymphs of the two species differ from one another by the form of the capitulum.

The examination of specimens of *R. sanguineus* s. l. from different parts of the world showed that both species exist in many different countries (Israel, France, Algeria, Senegal, Turkey, Yugoslavia), but the exact world distribution of the two species is still to be studied.

The importance of determining the distribution of the two species in the Western Hemisphere is obvious. Recently, during a visit to the Rocky Mountain Laboratory, Hamilton, Montana, we were able through the kindness of G. M. Kohls, to examine their collection of *R. sanguineus* from U. S. A.

Ticks from 17 States were examined in the time available. Forty-two females and numerous larvae and nymphs were from dogs. Five females were from deer, one nymph from a man, ten females and numerous nymphs had been found in houses, and five females were from an unknown host. All these ticks were exclusively *R. sanguineus* s. str.

The above material, though not sufficiently extensive to permit a rigorous generalization, suggests that *R. sanguineus* s. str. is probably the only one of the two above mentioned closely-related species present in the U. S. A.—B. Feldman-Muhsam, *The Hebrew University, Jerusalem.*

A CORRELATION OF MALPIGHIAN TUBULE SIZE WITH DEVELOPMENT OF *DIROFILARIA IMMITIS* IN THE MOSQUITO

Casual observations during routine dissections of many mosquitoes infected with *Dirofilaria immitis* suggested a possible correlation of Malpighian tubule diameter and parasite development. Measurements of tubule diameter were made on *Aedes aegypti, A. albopictus, Culex quinquefasciatus,* and *Anopheles quadrimaculatus.* After the tubules had been dissected out into normal saline on a glass slide, a plastic cover slip was placed over them and an excess of saline then run under the slip to prevent distortion of the tubules. The average diameter of five tubules per female of 100 females of each species was determined together with a record of those females exhibiting normal development of the parasite. Portions of the tubules obviously being enlarged by filarial growth were avoided in the measurements. Females from a single batch always were used to insure uniformity.

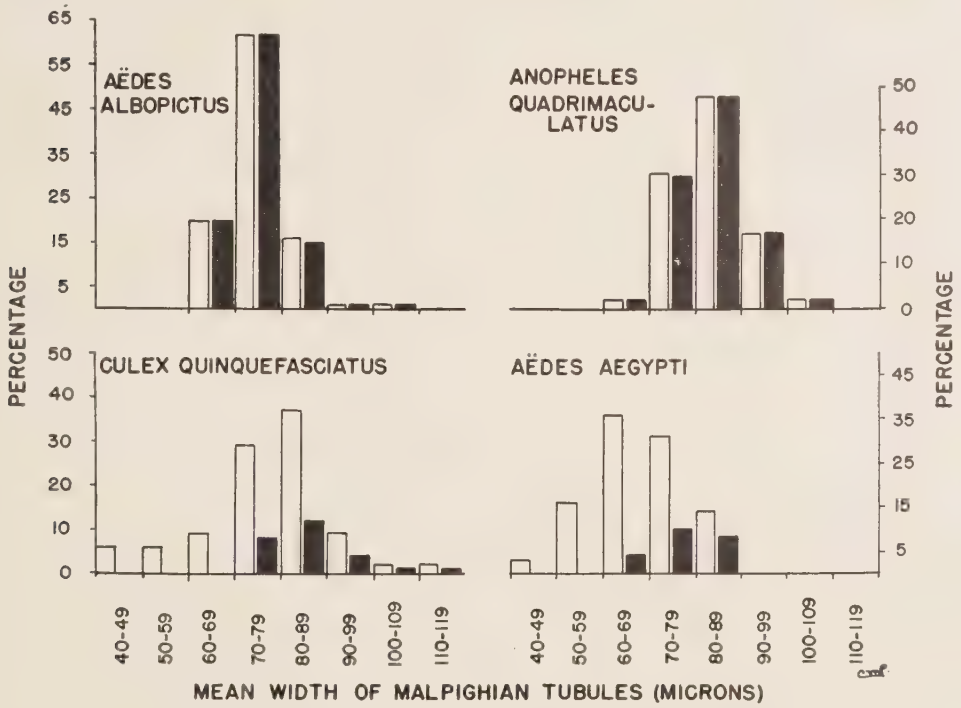


FIG. 1. Relation of Malpighian tubule diameter to development of *Dirofilaria immitis* in several species of mosquitoes. □—percentage of females with indicated tubule diameter. ■—percentage of females showing development of the parasite.

The data for these observations are presented in Figure 1. Although 93 females of the 100 *A. aegypti* dissected showed unchanged microfilariae in their tubules and thus may be classified as positive, the data presented here are concerned only with mosquitoes exhibiting larval development of the parasite and this accounts for the many negative females recorded for this species.

These observations suggest that mosquitoes with predominantly wide tubules showed a preponderance of normal development of *D. immitis* larvae, whereas those with tubules ranging into narrower diameters exhibited parasite development mainly in individuals with the wider tubules. However, it cannot be asserted that Malpighian tubule diameter is here shown to determine microfilarial transformation to the larval stages. If such were the case, it would be expected that a majority of microfilariae would initiate development in *A. aegypti* females possessing tubules of comparable size to those in *A. albopictus* or the anopheline. In the case of *Culex quinquefasciatus,* microfilariae reaching tubules of larger diameters initiated development. However, a considerable portion of the females with larger tubules were negative, and all of the females with smaller tubules were negative. Although it has been shown that great numbers of microfilariae are destroyed in the midgut of this species (Kartman, Exper. Parasit.

2: 27–78, 1953) this would not account for the fact that all of the *quinquefasciatus* females with small tubule diameters were negative for the parasite. Many microfilariae penetrate *aegypti* tubules having widths equally as narrow. Thus, although it seems clear that factors other than tubule size *per se* are undoubtedly operative, the present correlation of tubule diameter with filarial growth may be an indication that larval growth is partially dependent upon the size of the cells composing the Malpighian tubules since it is known that the microfilariae penetrate these cells before initiating further development.—Leo Kartman, *The Johns Hopkins University, School of Hygiene & Public Health, and the U. S. Public Health Service, Communicable Disease Center, Atlanta, Georgia.*

A METHOD OF EXPOSING FLIES TO INFECTIOUS MATERIAL

In the course of studies on the transmission of human intestinal protozoan parasites by means of the common house fly, a method of feeding cysts to flies was devised which might find application in other problems where test meals are used. This method was perfected in order to be certain the flies took the test meal in a minimum of time and without undue contamination of their exteriors. It is simple, requires little time, uses common materials and does not appreciably harm the flies. The latter is of particular importance if flies are to be released for flight. It was found that about 100 per cent of flies handled by the new method would feed to repletion within 20 minutes as compared to approximately 25 per cent which would feed in the same length of time if free within a cage.

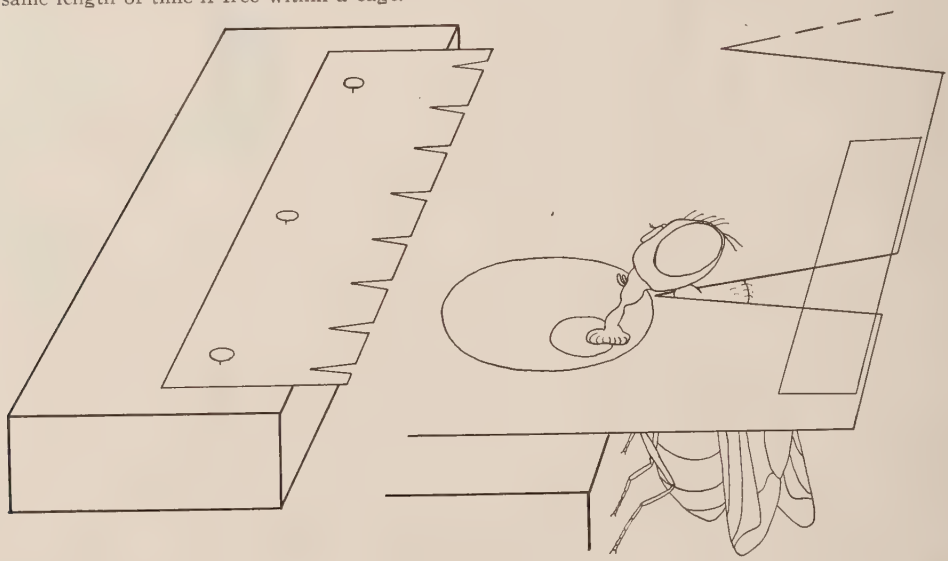


Fig. 1. The diagram to the left shows the apparatus with the notched paper tacked to the block of wood. The diagram on the right shows a fly secured in a notch by a piece of cellophane tape and feeding upon material placed on a cover slip.

Flies are placed in a retaining device constructed as follows: A series of V-shaped notches about one-half an inch apart are cut with small scissors along the edge of a piece of thin stiff paper (Figure 1). The common 3×5 file card is excellent paper to use. The notches are approximately 2 millimeters wide at the base and 6 millimeters long; however, the size is not critical and a few trials will produce the proper shape of the notches. The paper is placed on top of a small block of soft wood about one inch thick and arranged so the notches extend in a horizontal plane slightly beyond the edge of the block as it rests upon a table. The paper is then fastened in position with thumb tacks. The flies are grasped by the legs or wings with small curved forceps and slipped into the notches so that their heads extend above and inward and their bodies below the paper. If the legs protrude above they are drawn below by means of a needle. The flies which are literally hung by their heads in the notches do not ordinarily escape but for safety against this event, a small strip of cellophane (Scotch) tape is placed over the opening of the notch.

In our studies a drop of suspension of the protozoan cysts was placed near the edge of a cover slip lying upon the paper and manipulated so that the drop was brought near to the fly's

mouthparts. It was occasionally necessary to probe the fly's head so that it faced toward the drop, but it was not necessary to manipulate the body of the fly, regardless of its position. Flies which were made hungry by starving, readily fed upon the suspension and when replete were removed to a holding cage. The flies behaved in a normal manner in flight after being released from the holder.

We have handled 30 to 50 flies at one time by this method, replacing the drops of the suspension as these were consumed by the flies. Modifications of this holder can be made for special purposes depending upon the requirements of the investigator.—ROBERT C. RENDTORFF, *National Institutes of Health, Laboratory of Tropical Diseases, Columbia, South Carolina.*

LEPTOPHYLLUM OVALIS BYRD AND ROUDABUSH, A SYNONYM OF *LEPTOPHYLLUM TAMIAMIENSIS* McINTOSH

The author has collected recently specimens of *Leptophyllum tamiamiensis* from the rectum of *Elaphe quadrivittata deckerti.* A study of these specimens indicates that *Leptophyllum ovalis* Byrd and Roudabush, 1939 is a synonym of *L. tamiamiensis* McIntosh, 1939.

Careful comparison of the descriptions of the two species shows that the difference in size between the two groups of specimens described is not as great as Byrd and Roudabush suggested. They stated that *L. tamiamiensis* barely exceeds 1 mm., while *L. ovalis* measures 1.78 mm. The measurement, 1.78 mm., however, represents the mean length; shorter specimens measure 1.45 mm. Thus, the smaller specimens of *L. ovalis* approach the length of 1.2 mm. given for *L. tamiamiensis.* The difference in length, therefore, is only 0.25 mm.

L. ovalis was also separated from *L. tamiamiensis* by the shape of the ovary, which is oval in *L. ovalis* and spherical in *L. tamiamiensis.* The writer's series of specimens show that either shape is possible.

In general, the specimens described as *L. ovalis* were longer than those described as *L. tamiamiensis* and in these longer specimens the metraterm was found to be shorter and more muscular than in *L. tamiamiensis.* This would appear to be the most significant difference between the two groups of specimens and a valid character for their separation if proved constant. However, the writer's material shows that in longer specimens—that is, those within the range described for *L. ovalis*—the metraterm may be as long and as weakly muscular as is the case in *L. tamiamiensis.*

Muscular organs such as the metraterm should be avoided as taxonomic characters since their length and apparent muscularity vary with the state of contraction of the organ.

The species were further separated on the basis of host and habitat, *L. ovalis* being described from the small intestine of *Natrix taxospilotes* and *L. tamiamiensis* from the rectum of *Agkistroden piscivorus.* The writer's specimens are from the rectum of a third host, *Elaphe quadrivittata deckerti,* indicating that there is no strict host specificity.

Accordingly, as there appear to be no constant characters separating these two species, the writer proposes that they be made synonymous under the name *Leptophyllum tamiamiensis* McIntosh, 1939, this name having priority over *L. ovalis* by five months.—GERHARD A. SCHAD, *Institute of Parasitology, McGill University, Macdonald College, P.Q., Canada.*

THE SNAILS, *PSEUDOSUCCINEA COLUMELLA* (SAY), NEW INTERMEDIATE HOSTS FOR THE LIVER FLUKE *FASCIOLA GIGANTICA* COBBOLD

Pseudosuccinea columella, identified by Dr. J. P. E. Morrison of the U. S. National Museum, Washington, D. C., has been found to be a new host for *Fasciola gigantica.* The snails were obtained as adults from a water-cress area in the Pauoa district of Honolulu where about 300 similar snails showed no evidence of larval fluke infection upon dissection. The snails were experimentally exposed to miracidia of *F. gigantica* and maintained at room temperature (24 to 27° C.) in large evaporating dishes containing tap water, small rocks, pieces of lettuce and honohono plants (*Commelina diffusa*). Under these conditions, 10 snails dissected 15 days after infection showed many rediae with daughter rediae. Forty-two days after infection, a large number of cercariae were noted emerging from the snails and encysting on vegetation and sides of the dishes. Thirty of these encysted metacercariae were fed to each of two young guinea pigs. Fifteen days later, at necropsy, small fasciolid flukes, 1.5 to 2 mm. long, were recovered from the liver of these animals. The eggs of *F. gigantica* used in these tests were taken from adult flukes collected from natural infected local cattle.

Previous to this report, *Fossaria ollula* (Gould), identified by Dr. J. P. E. Morrison, was the only snail known in the Hawaiian Islands to be able to serve as a carrier of *F. gigantica* (Alicata, 1938, Hawaii Agr. Exp. Sta. Bul. 80, 22 pp.). Recently, this species has been listed by Hubendick (1952; Occas. Papers, Bernice P. Bishop Mus. 20 (19) : 307–328) as *Lymnaea volutata* Gould. The occurrence of *P. columella* is new to the Hawaiian Islands. This snail

possibly was introduced from the continental United States. It has been collected by the writer in the above mentioned water-cress area and in a stream (Pauoa) which joins this water-cress area.—Joseph E. Alicata, *University of Hawaii Agricultural Experiment Station, Honolulu.*

SIMULTANEOUS *TRICHURIS* AND *ASCARIS* INFECTION

In a paper entitled *Multiple Helminthic Infections,* Walter Koch & Heinz Steinetz (J. Parasit. 37: 307–308; 1951), have reported a "highly significant" discrepancy between the number of cases of simultaneous infection with *Trichuris* and *Ascaris* actually observed, and the number of cases of simultaneous infections expected—the latter figure based upon a probability computation by the writers, who state that the rate of double infection should be the product of the *Ascaris* rate times the *Trichuris* rate. In the material of Koch and Steinetz the number of double infections found is twice as high as anticipated. As to this difference the writers could give no explanation.

An infection with *Trichuris* or *Ascaris* is not purely accidental. The distribution of the cases, whether *Trichuris* or *Ascaris,* therefore, will not be directed exclusively by chance, but chance will apply directly to their distribution within boundaries set by the circumstances. Whether or not a person may be infected depends upon the presence of mature helminth eggs and upon the habits of the person in question as to hygiene, gardening methods, and food treatment.

The coincidence of *Trichuris* and *Ascaris* invasion is generally influenced by the biological similarities of these two helminth species as regards the dispersion and hatching of their eggs in the soil as well as by their way of invasion. As a consequence of the conformity of sources and ways of infection, simultaneous infections with *Trichuris* and *Ascaris* may well be expected to occur with a higher rate of coincidence than can be calculated stochastically. This explains, at least partially, why the observations of Koch and Steinetz did not conform to their statistical evaluation of the case. If the biological similarities between the two helminth species were unknown, it should be possible from reliable findings to deduce such similarities as a probable cause.—Johannes Harbo, *15 Falkoner Allé, Copenhagen, Denmark.*

SOME HELMINTH PARASITES OF ALASKAN BEAVERS

In connection with parasitological investigations conducted within the Territory of Alaska by the Animal-borne Disease Branch, Arctic Health Research Center, 56 beavers have been examined. This collection, made over a period of four years, covers a wide geographical area, encompassing the following general localities: Kalgin Island (16); Anchorage (4); Lake Louise (24); Fairbanks (6); and Arctic Village (6).

Forty-four (79 per cent) of the total number examined were infected with one or more of three helminth species; 20 harbored only one species, 21 harbored two species, and three harbored three species.

Stichorchis subtriquetrus (Rud., 1819) (Trematoda: Stichorchinae) was recovered from the ceca of eight beavers (14 per cent), with a maximum number of 204 specimens in a single animal. *Castorstrongylus castori* Chapin, 1925, (Nematoda: Strongylinae) was recovered from the small intestine of 20 beavers (36 per cent), with a maximum of 41 specimens. *Travassosius americanus* Chapin, 1925, (Nematoda: Trichostrongylinae) was recovered from the stomachs of 34 beavers (6 per cent), with a maximum of over 1000 specimens. These three helminth species do not seem to be limited geographically, since beavers from all localities were infected by them.

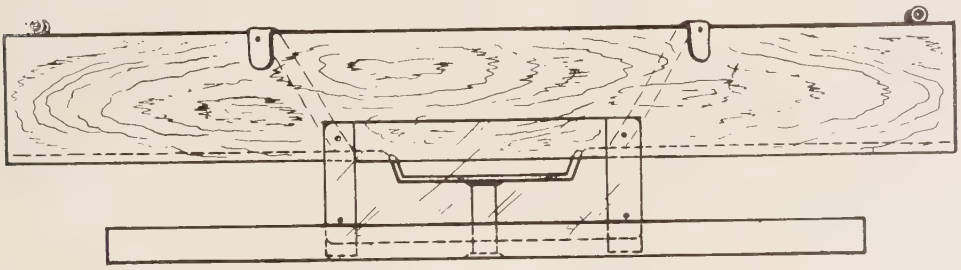
The results of this Alaskan investigation closely parallel the findings of Erickson (1944; Amer. Midl. Nat. **31:** 625–630) in Minnesota, who reported only one additional species, *Stephanoproraoides lawi* Price, 1934, from 140 beavers examined. Erickson also listed four other helminths, previously reported from this host. (*Paramphistomum castori* Kofoid and Park, 1937, he regarded as synonymous with *S. subtriquetrus.*) *Capillaria hepatica* (Bancroft, 1893), from the liver of a beaver that died in the National Zoological Park, was reported by Chitwood (1934; Proc. Helm. Soc. Wash. **1:** 10). This record was omitted in the list of species given by Erickson.

Orloff (1941; C. R. Acad. Sci. URSS. **31:** 641–643) investigated parasitism of beavers on the Voronezh preserve and found that 93.7 per cent of those on a farm and 80 per cent of those in natural habitats were infected with helminths. Concerning the pathogenicity of these helminths, he wrote, "The loss of the lot of beavers which perished during summer was due to helminthic invasions; these were represented by ascardosis, capillariosis of the liver, psilotrematosis—an invasion new to beavers, and, finally stichorchosis. The latter called forth the majority of loss (31%) among the beavers inhabiting the preserve."

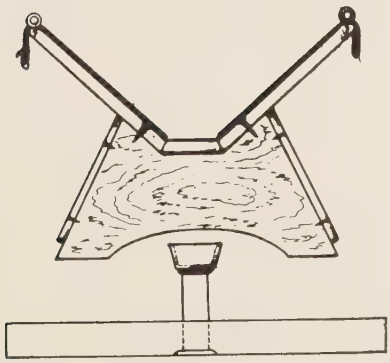
No pathological conditions attributable to helminth parasites were observed in the Alaskan beavers.—Bert B. Babero, *Animal-borne Disease Branch, Arctic Health Research Center, Public Health Service, Anchorage, Alaska.*

A BOARD DESIGNED FOR EXPOSING LARGER ANIMALS TO SCHISTOSOMES IN THE LABORATORY

Mice are the animals most commonly used in laboratory experiments on the human schistosomes. On occasion it is necessary to expose larger animals to the cercariae. In such experiments it is desirable to allow the cercariae to come in contact with the skin of the animal without extensive wetting, to protect the technician from exposure to the cercariae. A restraining apparatus for this purpose has been devised and used successfully with the agouti. It would also be useful in experiments on cats, dogs, monkeys, and similar animals.



A. SIDE VIEW
ASSEMBLED IN PLACE



B. END VIEW
EXPLODED

The upper portion of this apparatus is a modified animal board. It is made in the form of an open-ended trough thirty inches in length, with a flat bottom two inches wide, and six inch sides sloping at an angle of 45 degrees (see figure). At the center, a six inch section of the flat bottom is cut away. The upper edges of this opening should be rounded and smooth. The trough is supported by legs fastened ten inches from the end. A sheet of plexiglass is placed across these on each side, the upper edge fitting against the trough and the lower edge about

one-half inch above the tips of the legs. Hooks are placed near the corners of the board for fastening the feet of the animal. Two straps are fastened at the base of the trough at the ends of the cut out section. These are fastened across the belly of the animal to hold its back into the opening. The hair can be clipped or shaved through the opening and the animal is ready for exposure to cercariae.

The bottom portion of the apparatus consists of a tray 18×24 inches with one inch sides, a pedestal in the center of the tray, and a shallow pan that fits into the opening of the board. The pedestal should be high enough so that when the upper section is placed on the tray, the pan is in the opening in the floor of the trough and its rim rests just below the rounded upper edge of the opening.

For exposure a cercarial suspension is placed in the pan and the board and animal are lowered into place. The legs of the trough and the plexiglass prevent the splashing of cercariae on the operator and at the same time he is able to see if the cercarial suspension overflows. With practice the correct amount of suspension can be ascertained to get good skin exposure and a minimal loss of cercariae. To prevent accidental exposure to cercariae that may be spilled as a result of violent movement of the animal, a small amount of disinfectant is placed in the tray. After exposure, the board can be lifted and set aside until the animal is dry. This allows the cercariae to use the surface film to assist in penetration and further eliminates danger of human infection. During this time the pan may be readily examined for any cercariae remaining in suspension. For cleaning, the suspension pan should be flooded with clear water and allowed to overflow into the tray. The water and the cercariae remaining in the pan pour into the tray as it is emptied into the sink so that all parasites come in contact with the disinfectant.—Donald L. Price, *Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington 12, D. C.*

ANOTHER *ASCARIDIA GALLI* IN HEN'S EGG

Several cases of *Ascaridia* from the hen's egg were reported by Hall (1925, J. Parasit. 12: 105). Cram (1933, Eastern States Cooperator 9: 8–9, 20, 22) reported the occurrence of this parasite in the egg-forming organs of the hen, thus offering an explanation for its occasional occurrence in eggs, and Hall (1945, Poultry Science 24: 496–498) has reported a case from New York State. More recently, Schell (1951, J. Parasit. 37: 326) has reported the findings of a 46 mm. male *Ascaridia galli* hanging from the side of an infant's mouth while the child was having its morning feeding of egg and Pablum.

A vial containing an apparently live but inactive female *Ascaridia galli* 72 mm. in length was sent to the University of Connecticut on March 19, 1953, by a Connecticut resident. The letter accompanying the vial indicated that the worm had been found in the white of a raw fresh egg and expressed concern for two babies should they consume uncooked eggs containing such a parasite.—Lawrence R. Penner, *Department of Zoology and Entomology, University of Connecticut, Storrs.*

DIROFILARIA SCAPICEPS FROM *SYLVILAGUS FLORIDANUS* IN NEW ENGLAND

Many surveys of parasites from cottontail rabbits have been made in this country in recent years but records of rabbit filariae are meager and filariae do not seem to have been reported from rabbits in New England.

Leidy (1886, Proc. Acad. Nat. Sci. Phila. 38: 308–313) found filarial worms which he named *Filaria scapiceps,* under the skin of the hind foot of *Lepus sylvaticus* (*Sylvilagus floridanus mallurus*). The locality was not given. According to Hall (1916, Proc. U. S. Nat. Mus. 50: 1–258) Leidy's worms were probably from Pennsylvania, at any rate from the United States. Hall also reported two other cases, one from *Lepus campestris,* locality not given, and the other from a collection by Douthitt from *Sylvilagus floridanus alacer* at Sulphur, Oklahoma. The parasites in this case were found under the skin of the lumbar region. Harkin (1927, Can. Field. Nat. 61: 113) found microfilariae in the blood of a rabbit from British Columbia, and Alicata (1929, J. Parasitol. 15: 287) recorded a previously unreported case of adults collected in 1916 from *Sylvilagus floridanus mallurus* at Woodford, Virginia, inside the tarsus. He also reported worms from inside the tarsus of a hind leg of what was probably the marsh rabbit, *Sylvilagus palustris,* from Kingston, North Carolina. Schwartz and Alicata (1931, J. Wash. Acad. Sci. 21: 298–301) described microfilariae from the blood of *Lepus washingtonii* from the State of Washington. MacLulich (1937, Univ. Toronto Studies, Biol. Series No. 43: 83–86) reported *Dirofilaria scapiceps* adults from *Lepus americanus* in Ontario.

Manweiler (1938, J. Mamm. 19: 379) gave the first account of this worm in *Lepus americanus phaeonotus* in Minnesota and reported infection of about one-third of all specimens of

snowshoe hares examined in the winters of 1936–37 and 1937–38, with an increase from October to February and then a decrease to July. Highby (1943, J. Parasitol. 29: 253–259) reported 23 cases of infection out of a total of 57 snowshoe hares examined in Minnesota, with incidence rates of 13 to 58 percent in samples from 3 geographic extremes of its range. Highby also demonstrated that certain species of *Aedes* mosquitoes can transmit the infection to domestic rabbits.

Since then Llewellyn and Handley (1945, J. Mamm. 26: 379–390) reported that Bell and Chalgren of the U.S.P.H.S. found *Dirofilaria* in a small percentage of the 75 cottontails sent from Virginia to them for parasitic examination. The worms were from the intermuscular fasicae of the hind legs except for one in the subcutaneous fasciae of the back just behind the shoulder.

Erickson (1947, J. Wildlife Mgt. 11: 255–263) reported 18 genera of helminth parasites from *Sylvilagus* and collected 15 different species of helminths from 97 cottontails examined for parasites, but even though the leg joints of 30 rabbits were carefully examined, the filarial worm, *Dirofilaria scapiceps* was not found. In studies reported by Clancy, Jungherr, and Sime (1940, J. Wildlife Mgt. 4: 162–168), filariae were not reported from any of the 342 cottontails examined for internal parasites, nor did Cheatum (1952, P-R Project 1-R Supp. E. N.Y.S. Cons. Dept.) list any in recording parasites from 937 cottontails examined.

We have recently obtained four cottontails which were apparently *Sylvilagus floridanus mallurus,* from South Coventry, Connecticut. Three of these had microfilariae in their blood and despite a rather careful search we were able to recover only a single pair of adults from the left rear foot near the Achille's tendon of the rabbit which was live-trapped on March 17, 1953. The other two showing microfilariae in their blood were taken on February 19, 1953, and March 14, 1953. We have identified these as adults and microfilariae of *Dirofilaria scapiceps* (Leidy, 1886) Railliet and Henry, 1911. We have also had opportunity to examine five adult worms removed by Mr. Wm. B. Foster from the hind leg of a cottontail which he collected at Upton, Massachusetts, on June 12, 1952. These too have been identified as *D. scapiceps.*

Although there has been considerable importation of cottontail rabbits and of some snowshoe hares into Connecticut from other states in the past with the possibility of some being infected with filariae, it is not unlikely that these worms have been parasitizing rabbits in Connecticut and Massachusetts for a great many years. Careful examinations of fresh blood smears and leg joints will probably reveal a fairly high incidence.—Lawrence R. Penner, Donald W. Dery, and Joseph L. Knuckles, *Department of Zoology and Entomology, University of Connecticut, Storrs.*

BOOKS AND MONOGRAPHS RECEIVED

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